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The efficacy of supplementary sonic irrigation using the EndoActivator® system determined by removal of a collagen film from an *ex vivo* model

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Abstract

Aim To evaluate the efficacy of sonic irrigation (EndoActivator®) using various polymer tips and power-settings in a stained collagen *ex-vivo* model.

Methodology Fifty human, straight single-rooted extracted teeth were prepared to size 40,.08 taper. The roots were split longitudinally; stained collagen applied to the canal surfaces, photographed and re-assembled. The canals were subjected to syringe without supplementary (Group 1, n = 10), or with supplementary sonic (groups 2–5, n = 10) irrigation. EndoActivator® tip sizes (size 15, .02 taper for groups 2 & 3, size 35,.04 taper for groups 4 & 5) and power-settings (Low for groups 2 & 4, high for groups 3 & 5) were tested. After irrigation, the canals were re-photographed and the area of residual stained-collagen was quantified using the UTHSCA Image Tool program (Version 3.0). The data were analysed using Wilcoxon signed rank test and General Linear Mixed Models.

Results Supplementary sonic irrigation using EndoActivator® resulted in significantly ($P < 0.0001$) less residual collagen compared with syringe irrigation only. Agitation of irrigant using the large EndoActivator® tip with high-power resulted in significantly less (22.4% – 29.5%) residual collagen compared to other combinations (large-tip/low-power $P = 0.001$; small-tip/low-power $P = 0.01$; small-tip/high-power $P = 0.04$). There was no significant difference amongst the latter three groups ($P > 0.5$).

Conclusions Supplementary sonic irrigation using the EndoActivator® system was significantly more effective in removing stained collagen from the canal surface than syringe irrigation alone. EndoActivator® used with large-tip (size 35, .04 taper) and high power-setting in size 40,.08 taper canals was more effective than other combinations.

Introduction

Removal of the bacterial biofilm from an infected canal surface is one of the most important roles of root canal irrigation (Gulabivala *et al.* 2010). It has been accepted that irrigation using a syringe and needle can only deliver the irrigant to approximately 1 to 1.5 mm beyond the needle opening (Boutsioukis *et al.* 2009). Manual or automated agitation of the irrigant aids both its apical penetration beyond the stagnation plane (Bronnec *et al.* 2010, Gulabivala *et al.* 2010) and removal of surface adherent layers, be they smear layer (Caron *et al.* 2010), debris (Jiang *et al.* 2012) or stained collagen (Huang *et al.* 2007, McGill *et al.* 2008). The latter, closely representing microbial biofilms (Abbott *et al.* 2011, alarab Mohmmed *et al.* 2016).

Manual-dynamic agitation of irrigant can be achieved using a file (Bronnec *et al.* 2010) or a tapered gutta-percha cone (Huang *et al.* 2007) but may be considered laborious and less effective than ultrasonic or sonic devices (Jiang *et al.* 2010a). Endovac™ is another device aimed at active irrigation and shows promising debris removal (Nielsen *et al.* 2007, Siu *et al.* 2010) although less-so for additional anti-bacterial efficacy (Townsend *et al.,* 2009 Miller *et al.* 2010). Ultrasonic irrigant agitation is effective (Lee *et al.* 2004, Van der Sluis *et al.* 2006, 2009, Jiang *et al.* 2010b, 2010c, 2011,), but may be accompanied by instrument fracture and dentine damage at 20–40 kHz (Boutsioukis *et al.* 2013), despite the use of a smooth wire designed for “passive ultrasonic irrigation” (Van der Sluis *et al.* 2005).

Sonic devices operate at lower frequencies (<200 Hz) and include the Vibringe® (Cavex Holland BV, Haarlem, The Netherlands) (Rödig *et al.* 2010) and EndoActivator® (Advanced Endodontics, Santa Barbara, CA, USA) (Ruddle 2007) systems.

The EndoActivator® is an electrically driven unit operating at stated frequencies of 33, 100 and 167 Hz (Ruddle 2007) but with measured vibrational frequencies of 160, 175 and 190 Hz, respectively (Jiang *et al.* 2010a). The instrument employs polymer tips of different sizes (size 15, .02 taper, size 25, .04 taper, size 35, .04 taper) to agitate irrigants (Jiang *et al.* 2010a) potentially avoiding the risks associated with ultrasonically-driven metal instruments.

The EndoActivator® does not create cavitation or acoustic streaming (Jiang *et al.* 2010a) but, compared with static or manual-dynamic irrigation, has been shown to have superior irrigant penetration into apical dentinal tubules (Paragliola *et al.* 2010), removal of debris, and breakdown of the smear layer (Caron *et al.* 2010). However, these merits were not evident when a small size 15, 02 taper tip was used (Klyn *et al.* 2010, Uroz-Torres *et al.* 2010, Merino *et al.* 2012).

Agitation of sodium hypochlorite (NaOCl) (Pasqualini *et al.* 2010, Bago *et al.* 2013), or chlorhexidine gluconate (Shen *et al.* 2010) solutions using the EndoActivator® system have been shown to produce synergistic bacterial load reduction (Pasqualini *et al.* 2010) as well as biofilm killing (Bago *et al.* 2013) and disruption (Shen *et al.* 2010). The EndoActivator® has also been shown to have similar results to laser-activated irrigation when removing *E. faecalis* from an extracted tooth model, with both systems more effective than conventional irrigation (Bago *et al.* 2013).

Previous studies investigating the efficacy of irrigant agitation have all used the EndoActivator® at maximum power-setting (10,000 cycles per minute) coupled with different tip sizes: size 25, .04 taper (Merino *et al.* 2012, Bago *et al.* 2013), size 15, .02 taper (Townsend & Maki 2009, Klyn *et al.* 2010, Uroz-Torres *et al.* 2010), or both (size 25, .04 taper; size 35, .04 taper) (Caron *et al.* 2010). The latter study did not clarify the protocol for tip selection and the influence of tip size was not analysed.

This study aimed to evaluate the efficacy of sonic irrigation (EndoActivator®) using different polymer tips and power-settings in a stained collagen *ex-vivo* model. The null hypotheses for the study were that irrigant agitation, using various tip-sizes of EndoActivator® at different power-settings, had no significant effect in the removal of stained collagen films from the canal surface.

Materials and Methods

Ethical approval was granted for the use of extracted teeth from the UCL Eastman Biobank (Study number: 1301). A power analysis for a two-sample proportions chi-squared test (б =

0.05, power = 90%, difference at the apical third = 20 percentage points) based on data from a previous study (Huang *et al.* 2007) for comparisons between the test groups (sonic irrigation) against the control group, indicated that a minimum of 9 root canals per group were required to detect a significant difference. However, there was a lack of prior equivalent data on sonic irrigation to estimate the sample size for comparisons amongst the four test groups.

Selection and preparation of teeth

Fifty extracted human permanent single-rooted teeth with straight, single canals, mature apices and free from dental caries or resorption, were collected and stored in 4% saline (CellPath, Newtown, UK). The teeth were decoronated using a diamond-coated disk (Abrasive Technology Inc., Westerville, OH, USA) to give a uniform working length of 18 mm to the apical terminus. The root canals were prepared to an apical size 40 and 0.08 taper using a combination of stainless steel files (Flexofile® Dentsply Sirona, Ballaigues, Switzerland) and nickel-titanium rotary instruments (ProTaper® and SystemGT®, Dentsply Sirona) in a 70:1 controlled-torque, low-speed rotary handpiece (TCM Endo III®, SybronEndo Corp, West Collins, Orange, CA, USA) at 300 rpm. Instrumentation was accompanied by standard, intermittent irrigation with 2.5% NaOCl (Teepol® bleach, Teepol products, Egham, UK); after each instrument, 3 mL NaOCl was delivered using a Monoject® syringe with a 27 gauge needle (Sherwood Medical, St. Louis, MO, USA). Each tooth was then embedded in silicone putty matrix (President Putty Coltène, Altstätten, Switzerland) to aid reassembly of the tooth following splitting.

The teeth were grooved longitudinally on the buccal and palatal surfaces using a diamond disc (Abrasive Technology Inc.), placed between 2 opposing scalpel blades (Size 11 blade, Swann-Morton, Sheffield, UK), which were inserted into the grooves and split into two halves with a mallet. Four even layers of collagen (Type I rat tail collagen in 0.6% acetic acid solution, First Link Ltd., Birmingham, UK) mixed with calligraphic ink (Kai-Ming, Tainan, Taiwan), in a ratio of 5:1, were painted on the canal surfaces. The solvent was allowed to

evaporate from the acid solution at room temperature for 48 hrs to allow the collagen to form a gel.

Each split half of the tooth was divided into apical, middle, coronal segments of equal lengths of 6 mm and marked (using a sharp pencil on the unpainted surface). Each pair of root halves was placed on a backlit radiograph-viewer and photographed (Fujifilm FinePix S2 Pro digital camera, Tokyo, Japan) in a standard fashion (6). The split teeth were then reassembled in the silicone putty matrix using ribbon wax to seal the gap between the two halves and randomly allocated to five experimental groups (n = 10 each) for syringe irrigation without supplementary (Group 1) or with supplementary sonic irrigation using EndoActivator® for irrigant agitation (Groups 2–5). Amongst groups 2–5, small-tip (size 15, .02 taper) with low power-setting was used for group 2, small-tip with high power-setting for Group 3, large-tip (size 35,.04 taper) with low power-setting for Group 4, and large-tip with high power-setting for Group 5.

Evaluation of oscillatory amplitude of EndoActivator tips

The oscillatory amplitudes of the EndoActivator® tips, in motion within air or water, were measured using an image-capture model that employed a mounted digital camera (CoolsnapPRO-cf, Media Cybernetics, Marlow, UK), with a capture-rate of 10 frames per second, connected to an imaging software package (Image-Pro Plus v4.5, Media Cybernetics, Marlow, UK). The EndoActivator® was mounted on a stand, with the tip edge adjacent to a calibrated metal ruler, and illuminated using a continuous wave focused light source (Schott KL1500 cold light source, Schott UK Ltd, Stafford, UK). Five representative images were captured of large and small tips running at high- and low-power settings within both air- and water-filled 5mL glass vials. The amplitude of oscillation was measured using the image analysis software (Image-Pro Plus v4.5) and mean amplitude calculated for each group (n = 5).

Irrigation experiments

The syringe irrigation protocol for Group 1 was adapted from a previous study (Huang *et al.* 2007). A total volume of 36 mL of 2.5% NaOCl was delivered from a Monoject® endodontic

3 mL syringe through a Luer-lock 27 gauge side-cut open ended needle (Sherwood Medical, St. Louis, MO, USA), at a rate of 1 mL sec^{-1}, in twelve 3 mL boluses. The needle tip was inserted to a maximum depth of 4 mm short of the canal terminus and moved, with 4 mm amplitude, in an apical-coronal direction away from this maximum depth. After every 9 mL of irrigant delivered, the irrigant was left in the root canal for 1 minute, giving a total of 10 minutes of NaOCl exposure time.

The irrigation protocol for the canals in Groups 2–5 was the same as in Group 1 with the addition of 1-minute irrigant agitation after every 9 mL of irrigant delivered. The designated small (groups 2 & 3) or large (groups 4 & 5) nylon tip was inserted to the apical terminus, and activated by the EndoActivator® at the designated low (groups 2 & 4) or high (groups 3 & 5) power-setting. New batteries (AA Duracell® alkaline, Geneva, Switzerland) were replaced in the EndoActivator® handpiece at the commencement of each sonic irrigation group test.

After completion of the irrigation regimen, the split teeth were disassembled and left at room temperature for 24 hours to allow evaporation of residual fluid. Digital images were taken as previously described.

Image analyses

The pre- and post-irrigation images of each tooth were loaded as paired JPEG format (1.4 MB) sets to facilitate measurement manipulation on Adobe Photoshop CS5® software (San Jose, CA, USA). On the pre-irrigation image, the “Line” Tool was used to draw a polygon around each 6 mm segment of the canal (coronal, middle, apical), taking care to follow the outline of the canal exactly. The “Magnetic Lasso” Tool was then used to highlight and separate the canal surface from the rest of the image and saved as a 256 Grey-scale mode separate layer. The “Magnetic Lasso” was also employed to separate the coronal-, mid- and apical thirds of the canal to facilitate analysis at a sectional level. The grey-scale has 256 values, which range from 0 (representing absolute black) to 255 (representing absolute white). This process was repeated for the post-irrigation images. The pair of pre- and post-irrigation grey-scale layers (identical in shape but different in grey-scale value) were

transferred to an analysis programme (UTHSA Image Tool, university of Texas Health Science Center, San Antonio, TX, USA) to quantify the proportion of canal surface coverage with stained collagen. The grey value of 45 was independently agreed-upon by three individuals, who held experience of the *ex vivo* stained collagen model, and employed as the threshold to stratify the entirety of the canal surface with presence (0-45 grey values) or absence (46+ grey values) of stained collagen (Figure 1). The number of 0-45 grey value pixels after irrigation for each third of each half of the split canal was divided by the respective number in the pre-irrigation image. This represented the proportion of canal surface coverage with residual stained collagen following irrigation.

Data analyses

Kolmogorov-Smirnov and Shapiro-Wilk tests for Normality were used to test the hypothesis that the percentage values of canal surface coverage with residual stained collagen did not fulfil the assumption of normal distribution. The percentage area of residual stained collagen coverage of canal sides A and B were compared using Wilcoxon signed rank test (STATA 12; STATA Corporation: College Station, TX, USA). A general linear mixed model was used to account for the clustering effect of the measurements taken from different levels of the same tooth (STATA 12) and to analyse the effects of the following potential factors on the efficacy of stained collagen removal: Irrigant agitation; EndoActivator® tip-size and power-setting; and the corono-apical segments of the root canal. The effects of tip size and power-setting were further analysed by including data from the groups 2–5 only. The proportion of canal surface coverage with residual stained collagen was used as the dependant variable.

Results

The amplitude of the two different tips within air or water whilst running at high and low power is detailed in Table 1.

The hypothesis that the percentage values of canal surface coverage with residual stained collagen was normally distributed was not rejected (P = 0.1). Paired t-test revealed no significant difference in the amount of residual collagen present on side A *versus* side B

of the canal (P = 0.07 for coronal thirds; P = 0.4 for middle thirds; P = 0.8 for apical thirds). The data from the two sides were therefore pooled for further analyses. There was substantially more residual collagen on canal surfaces following syringe irrigation without supplementary sonic irrigation (93.8%±9.5% to 97.3%±3%) compared with those canals exposed to sonic irrigation (27.9%±18.1% to 83.5%±14.6%) (Figure 2).

The general linear mixed model (Table 2, model 1) revealed that "mode of irrigation" (P < 0.0001) and "corono-apical level of canal" (P = 0.01) had significant association (Table 2) with the percentage of canal surface coverage with residual stained collagen following irrigation. Syringe irrigation without supplementary sonic irrigation resulted in significantly (P < 0.0001) more residual collagen than sonic irrigation using the EndoActivator®, regardless of tip-size and power-setting (Table 2). Following irrigation, the apical (coefficient = -6.7; 95% CI -11.7, -1.6) and middle thirds of the canal displayed significantly less residual collagen than the coronal thirds (coefficient = -8.8; 95% CI -14.0, -3.7) (Table 2, model 1). . There was no significant (P = 0.5) difference between the middle and the apical thirds.

The general linear mixed model (Table 2, model 2), incorporating the data from groups 2 to 5 only, revealed the EndoActivator® tip-size and power-setting combination had a significant (P < 0.0001) influence on its efficacy. Agitation of irrigant using the large-tip/high-power (group 5) resulted in significantly less residual collagen than using small-tip/low-power (group 2) (coefficient = 22.4; 95% CI 4.1, 40.7), small-tip/high-power (group 3) (coefficient = 29.5; 95% CI 11.3, 47.8) or large-tip/low-power (group 4) (coefficient = 27.0; 95% CI 8.7, 45.3). There was no significant difference amongst the latter three groups (P > 0.5) (Results not shown).

Discussion

The *ex vivo* test model adopted from (Huang *et al.* 2007) has been judged suitable for investigation of root canal irrigation parameters as it allows progressive degradation of the measured substrate in a manner similar to artificial root canal bacterial biofilm (Abbott *et al.* 2011). A recent study investigating the percentage of *E. faecalis* biofilm removal from 3D

printed photopolymer root canals revealed 89% removal from the surface after irrigation with 9 mL of 2.5% NaOCl and irrigant agitation using EndoActivator® (alarab Mohmmed *et al.* 2016). The present *ex-vivo* study reported a much lower efficacy with a maximum of 72% collagen film removal from the apical canal surface using the large tip and high power-setting, whilst the minimal removal was 16% from the coronal third using large tip but low power-setting. The high efficacy reported by alarab Mohammed *et al*. (2016) may be attributed to the simple canal anatomy with smooth polymer canal surface. Nevertheless, the above studies support the validity of the use of collagen film as a bacterial biofilm simulant in an *ex vivo* model for initial investigation of irrigation devices.

The length and size of canal preparation, as well as the syringe irrigation protocol were adapted from previous studies (Huang *et al.* 2007, McGill *et al.* 2008) to allow comparison. The dimensions of canal preparation (size 40;.08 taper) provided sufficient space for both the irrigant needle and large sized tips. Although this large dimension may be considered to violate the principle of dentine conservation, the apical size was consistent with the apical foramen diameter of maxillary incisors associated with periapical lesions (Gesi *et al.* 2014).

The syringe irrigation protocol was modified (Huang *et al.* 2007) in two respects, to bring the irrigation protocol closer to clinical reality, as follows: (1) a gauge 27 side-cut open-end needle was used instead of gauge 30 with a close-end side-open tip design; (2) the needle was moved apico-coronally during irrigation instead of fixing it 4 mm from the apical terminus. The surface coverage with residual collagen film following various irrigation groups might have been over-estimated when compared with clinical reality as a proportion of the canal surface would have been mechanically debrided during enlargement (Peters *et al.* 2001). However, the coating of the entire canal surface after enlargement controlled the confounding effect due to the variability of extent of surface touched by the instrument.

The syringe irrigation protocol removed less stained-collagen in the apical third than the range reported (Huang *et al*. 2007 or McGill *et al*. 2008). In addition, the present study revealed minimal difference amongst the corono-apical thirds following syringe irrigation, in contrast to the significantly less residual collagen present in the apical than the coronal third

reported by Huang *et al*. (2007) and McGill *et al*. (2008). The difference in these findings may be attributed to the adjusted irrigation protocol that applied vertical reciprocation of the needle tip as opposed to static positioning, 4 mm from the apex. The needle penetration (4 mm from canal terminus) in this study was consistent with clinical practice, where it may range between 2-5 mm from the working length (Kong 2014). Computational irrigant flow studies (Boutsioukis *et al.* 2010) that employed a model of similar dimensions to this study (size 45,.06 taper) indicate that reduced apical fluid pressure and shear stresses occur when the needle is moved further from the working length. The observed superior collagen layer removal at the site of irrigant deposition agrees with previous findings (Huang *et al.* 2007, McGill *et al.* 2008).

Despite the improved needle irrigation performance in this study, the efficacy of collagen film removal was distinctly enhanced by additional agitation using the EndoActivator®, regardless of coronal-apical level, or EndoActivator® tip size or power-setting. The null hypothesis for the study was rejected.

The superior efficacy of dynamic-agitation of irrigant compared with syringe irrigation without agitation (albeit with a push-pull movement) was confirmed and was consistent with expectations based on previous studies (Huang *et al.* 2007, McGill *et al.* 2008). These findings were also in keeping with other outcome measures evaluated for the EndoActivator®, including irrigant penetration to working length (Merino *et al.* 2012) and into dentinal tubules (Paragliola *et al.* 2010), smear layer removal (Caron *et al.* 2010), and *E. faecalis* biofilm removal (Bago *et al.* 2013).

This study revealed that the combination of EndoActivator® large-tip with high power-setting was significantly more effective in collagen film removal compared to other combinations. Although Jiang *et al.* (2010a), had compared small (size 15,.02 taper) and medium (size 25,.04 taper) EndoActivator® tips vibrating at 190 Hz for irrigant agitation and had found no significant difference in debris removal, their study was not able to test the efficacy of the large (size 35,.04 taper) tip due to their smaller canal preparation (size 30,.06 taper).

However, there are insufficient selection of tips with varying tip size and taper for further investigation of their interacted effects in different canal dimensions.

The superior efficacy of the large-tip (size 35,.04 taper) and high power-setting may be theoretically attributable to two factors: (1) increased direct mechanical removal through increased canal wall contact; or, (2) increased energy applied to the irrigant as a result of greater tip rigidity. The first hypothesis was rejected in a separate study, in which the EndoActivator® had negligible mechanical effect of on stained collagen, in the absence of irrigant (Gazani 2016). Furthermore, the vibration amplitude of the large tips was smaller than that of the small tips, regardless of power-setting or medium of immersion. The second hypothesis, suggesting higher hydrodynamic shear stresses is plausible, given the improved irrigant penetration (Merino *et al.* 2012), and fluid exchange within the root portions (Boutsioukis *et al.* 2010). The potentially closer proximity between the larger tip and collagen layers may also synergise the effect.

The present findings are strongly suggestive that use of high power-setting in combination with the largest *fitting* tip according to the canal dimension may optimise the efficacy of active irrigation when using the EndoActivator®. Ultimate verification may emerge from appropriate human randomised controlled trials. The findings do not however imply that all canals should routinely be prepared to apical size 40,.08 taper. Canal enlargement should be guided by all the factors clinicians would normally apply in judging the enlargement required to facilitate irrigant and root filling material delivery.

Conclusions

Supplementary irrigant agitation using the EndoActivator® was significantly more effective in removing stained collagen from *ex vivo* root canal walls, prepared to size 40,.08 taper, than syringe irrigation only. Sonic irrigation using the EndoActivator® system was significantly more effective when a large tip (size 35, .04 taper) with high power-setting was used.

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Conflict of Interest statement

All authors report grants from Dr Cliff Ruddle during the conduct of the study.

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Figure Legends

Figure 1 Image series displaying different Grey Scale Values for depiction of "cleaned" or "uncleaned" root canal surface area (Grey Scale of 45 chosen as ideal representative value)

Figure 2 Mean (±SD) percentages of canal surface coverage with residual collagen following irrigation by experimental group and corono-apical thirds of canal.

Table 1 Amplitude of sonic tip

Power Setting	Large Tip 35 ISO/0.04 taper	Small Tip (15 IS)/ 0.02 Taper)
	Amplitude (mm)	Amplitude (mm)
Slow in air	1.05	1.50
Fast in air	1.50	1.55
Slow in fluid	0.75	1.00
Fast in fluid	0.90	1.10

Table 2 Generalized linear model incorporating "mode of irrigation" and corono-apical level of canal" as independent variables and "percentage of canal surface coverage with residual stained collagen" as the dependent variable.

Independent variables	Coefficient	95% CI for coefficient	*P*-value	Z-value
Model 1 (full dataset was included)				
Mode of irrigation			*<0.0001	
Static irrigation (Reference)	0	-	-	-
EndoActivator® (small tip, low power)	-33.1	-49.4, -16.7	<0.0001	-4.0
EndoActivator® (small tip, high power)	-25.9	-42.3, -9.5	0.002	-3.1
EndoActivator® (large tip, low power)	-28.4	-44.8, -12.0	0.001	-3.4
EndoActivator® (large tip, high power)	-55.5	-71.9, -39.1	<0.0001	-6.6
Corono-apical level of canal			*0.01	
Coronal (Reference)	0	-	-	-
Middle	-8.8	-14.0, -3.7	0.003	-3.4
Apical	-6.7	-11.8, -1.5	0.01	-2.6
Random effect parameters	Estimate	92% CI for Estimate		
Variance for each tooth	293.1	183.4, 468.5		
Variance for each observation	339.7	285.1, 404.8		
Model 2 (data from groups 2-5 were included)				
Mode of irrigation			*<0.0001	
EndoActivator® (large tip, high power)	0	-	-	-
EndoActivator® (small tip, low power)	22.4	4.1, 40.7	0.02	2.4
EndoActivator® (small tip, high power)	29.5	11.3, 47.8	0.002	3.2
EndoActivator® (large tip, low power)	27.0	8.7, 45.3	0.004	2.9
Corono-apical level of canal			*0.01	
Coronal (Reference)	0	-	-	-
Middle	-11.6	-17.5, -5.3	<0.0001	-3.7
Apical	-9.2	-15.5, -3.0	0.004	-2.9
Random effect parameters	Estimate	92% CI for Estimate		
Variance for each tooth	367.4	218.4, 618.1		
Variance for each observation	406.0	333.7, 493.9		

* *P*-value for test of heterogeneity for categorical variable

Figure 1: Image series displaying different Grey Scale Values for depiction of "cleaned" or "uncleaned" root canal surface area (Grey Scale of 45 chosen as ideal representative value)

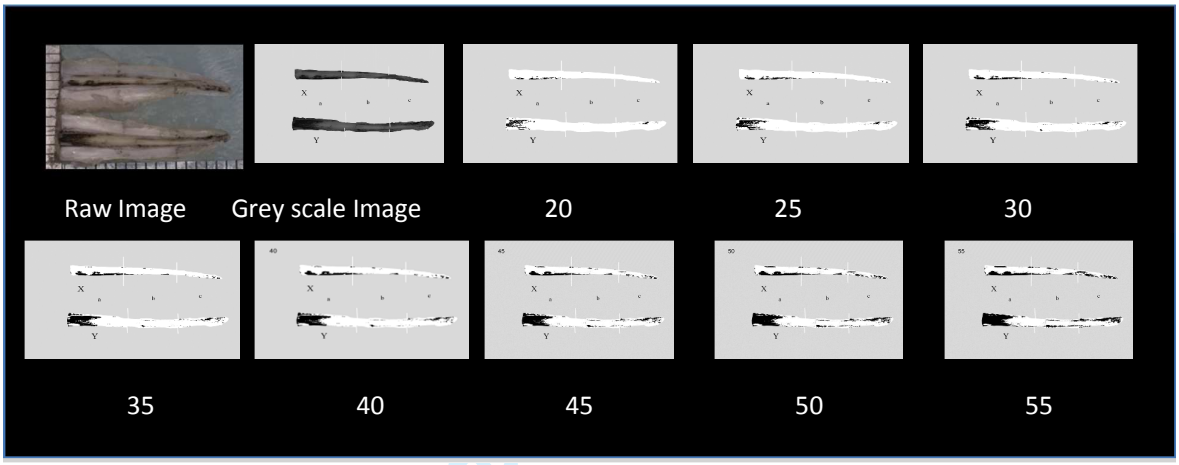

Figure 2: Mean (±SD) percentages of canal surface coverage with residual collagen following irrigation by experimental group and corono-apical thirds of canal.