

Evaluation of Smear Layer: A Comparison of Automated Image Analysis versus Expert Observers

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Abstract

Consistent and reproducible evaluation techniques of the smear layer in root canals in scanning electron microscopy studies are needed when comparing various instruments and techniques. In this study, the performance of 3 experienced blinded evaluators applying the Hulsmann technique was compared with a digital analysis method. Smear layer in the apical third of root canals of 35 freshly extracted teeth prepared by using nickel-titanium rotary instruments, Er:YAG and Er,Cr:YSGG lasers was scored on coded images. There was good agreement between the digital analysis method and the different evaluators (kappa analysis) across the range of the Hulsmann scores. Image analysis might be useful for evaluating the degree of smear layer removal in endodontic research. (*J Endod* 2008;34:999–1002)

Key Words

Dentinal tubules, digital imaging, lasers, smear layer

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Smear layer has been defined as a layer of debris on the surface of dental tissues created by cutting a tooth. It varies in thickness, roughness, density, and degree of attachment to the underlying tooth structure, according to the instruments and materials used, with some techniques that use irrigants such as ethylenediaminetetraacetic acid (EDTA) recognized to give effective removal of smear layer (1).

A smear layer might partially or completely occlude dentinal tubules, and the bacteria, endotoxins, and debris contained within it could contribute to ongoing periapical inflammation. Moreover, as well as containing bacteria, smear layer might prevent medicaments from adequately diffusing from the root canal space into the dentinal tubules (2). For these reasons, complete removal of the smear layer is seen as desirable, and accordingly, irrigants such as EDTA (3), sodium hypochlorite (4, 5), chlorhexidine gluconate (6), organic acids, MTAD (Biopure; Dentsply Tulsa Dental, Tulsa, OK) (7), and combinations of these are used clinically (8, 9). Physical techniques for smear layer removal include ultrasound (endosonics) and pulsed middle infrared lasers, both of which cause cavitations and pressure waves within the root canal space (8, 10).

Comparing the effectiveness of smear layer removal methods typically involves scoring high magnification (1000×) photomicrographs from scanning electron microscopy (SEM) studies, particularly of the apical third of the root canal. The images are coded and then scored by blinded evaluators by using qualitative or semiquantitative scales such as those described by Prati et al. (11) and Hulsmann et al. (12), with the latter the most commonly used. Other methods have involved tracing SEM photomicrographs onto graduated tracing paper for subsequent measurement (13) and using resin replicas of the surface under examination (14).

The use of digital image analysis methods in dentistry is becoming more popular, with reports of its use for assessing the curvature of root canals (15), dentin removal during root canal preparation with various techniques (16), and the efficiency of obturation methods (17). In 2007, Giocca et al. (18) described a computerized automated analysis technique for counting dentinal tubules; however, the use of this method for characterizing a prepared dentinal surface has not been evaluated.

The purpose of the present study was to validate an image analysis method for evaluating smear layer removal, comparing this with the well-established gold standard of the ordinal scoring system with 3 observers of Hulsmann et al. (12).

Materials and Methods

A total of 35 single-rooted extracted human teeth had their crowns removed at the cemento-enamel junction. The roots were randomly allocated into 5 groups of 7 each as follows. The positive control groups (groups 1 and 5) were prepared by using K3 files (Sybron Endo, West Collins, CA) in a 4:1 reduction handpiece (WE-66 EM; W & H, Buermoos, Austria), by using the variable taper, variable tip-size technique recommended by the manufacturer. In group 1 (control for smear layer removal), irrigation with 1% sodium hypochlorite and 15% EDTAC (1 mL of each) was used between the introductions of files. In group 5 (control for the presence of smear layer), only water was used for irrigation, ensuring a smear layer was present.

Samples in group 2 were treated with an Er:YAG laser (KEY3; KaVo, Biberach, Germany), with a fiberoptic endodontic handpiece with 200-, 320-, and 400- μ m diameter fibers, at panel settings of 500, 450, and 450 mJ, respectively, at a pulse frequency of 4 Hz. The Er:YAG laser was used in an experimental technique developed by Rutley (19). In brief, the coronal two thirds of the root canals were treated sequentially

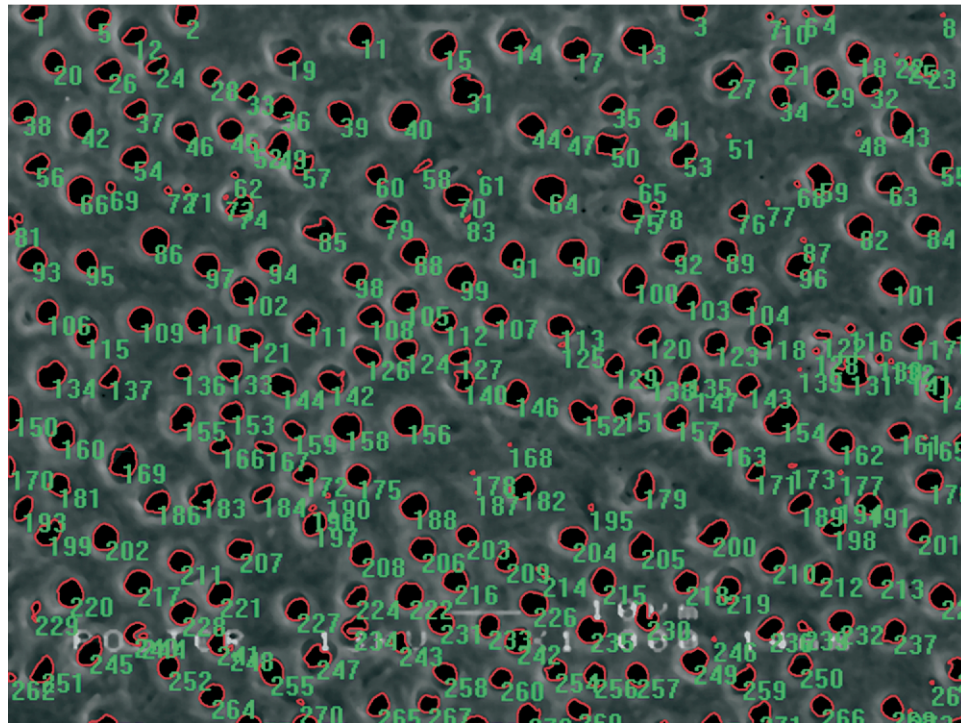


Figure 1. SEM of a group 1 specimen after digital image analysis. Boundaries of tubules are highlighted. Numbers indicate individual tubules. Original magnification, $\times 1000$.

with 400-, 320-, and 200- μm fibers. The apical third was initially prepared with K files by using balanced force technique to a size 30K file and then lased by sequential use of 200-, 320-, and 400- μm fibers, stepping back in 1-mm increments between successively larger fibers. The canals were irrigated after instrument pass with copious amounts of normal saline.

Group 3 samples were treated with an Er,Cr:YSGG laser (Waterlase MD; Biolase Technology, Irvine, CA) at a panel setting of 1.5 W with 24% water flow and 34% air flow, with 200-, 320-, and 400- μm diameter optical fibers (Z2, Z3, and Z4, respectively), as recommended by the manufacturer. Samples in group 4 did not undergo endodontic treatment and served as negative controls.

To assess the degree of smear layer removal, the roots were first dried and grooved longitudinally and split into 2 portions by wedging a fine chisel into the groove and then carefully twisting the chisel. This prevented the chisel going through the specimen and forcing debris into the canal. After the roots in group 4 were split, loosely bound pulp soft tissue remnants were removed by using blasts of compressed air.

SEM Examination

The split root samples were critical point dried, sputter-coated with platinum, and examined with an SEM (JEOL 6400; Tokyo, Japan) at 15 kV and at a final magnification of 1000 \times . The fields selected were all in the apical third region and were recorded at the middle of this region to show representative areas, with no attempt to select images showing any particular feature (such as open tubules) (Fig. 1). Images were recorded in lossless digital TIFF format. The images were balanced for gamma, contrast, and brightness before being used for analysis.

Evaluation of SEM Images

The assessment criteria described by Hulsmann et al. (12) were used to assess the presence of smear layer, but the order of the index was reversed such that the higher the score, the better the degree of

smear layer removal. A total of 84 coded images from samples in groups 1–4 were evaluated by 3 evaluators who had been trained to assess by using the published assessment criteria immediately before the commencement of the evaluation. Images were projected onto a large screen in a darkened lecture hall. The evaluators were instructed to apply the Hulsmann criteria strictly and were given copies of the article by Hulsmann et al. Each evaluator gave an independent score without reference to other evaluators. The scores were then tallied.

To establish the effectiveness of the digital imaging technique, the 5 reference images from the article by Hulsmann et al. (12) were first processed by using Image Pro-Plus (Media Cybernetics, Bethesda, MD) to enhance the edges. To determine the percentage of the total area occupied by the lumen of dentin tubules, the pixels associated with the tubules were counted. The resulting 5 percentage values were then used as a baseline measurement for each of the respective 5 scores (Table 1). With this approach, a total of 105 SEM images were then analyzed, and a score was assigned according to the percentage cutoffs. In cases in which the analysis software failed to automatically recognize the boundaries of the tubules, the outline was corrected manually by using a

TABLE 1. Hulsmann Assessment Standard Image Percentage Cutoffs and Overall Agreement in Scores

Score	Hulsmann Images Percentage Cutoffs	Agreement Between Hulsmann Evaluators and Digital Technique	
		Fleiss' Kappa	Agreement
1	<0.16%	0.795	Good
2	0.17%–0.49%	0.598	Moderate
3	0.50%–1.24%	0.759	Good
4	1.25%–5.31%	0.831	Very good
5	>5.32%	0.843	Very good
Overall		0.779	Good

TABLE 2. Interexaminer Differences

	Weighted Kappa	Agreement
Evaluator 1 vs 2	0.926	Very good
Evaluator 1 vs 3	0.876	Very good
Evaluator 2 vs 3	0.952	Very good
Evaluator 1 vs digital	0.790	Good
Evaluator 2 vs digital	0.834	Very good
Evaluator 3 vs digital	0.818	Very good
Digital initial scores vs digital scores after 3 months	0.973	Very good

graphics tablet. Any regions of debris incorrectly identified as tubules were excluded from analysis.

Statistical Analysis

The Fleiss' kappa analysis was used to compare agreement between the evaluators and the digital method. Because the Fleiss' kappa method does not evaluate the difference between individual groups, a weighted Cohen's kappa analysis was necessary to evaluate agreement between each of the 3 evaluators and the digital scoring method. The weighting was required because the Cohen's kappa test does not take into account the degree of disagreement between observers and rates all levels of disagreement equally (20). The following guidelines have been proposed for interpreting kappa (21): $\kappa \leq 0.20$ indicates poor agreement; $0.21 \leq \kappa \leq 0.40$ indicates fair agreement; $0.41 \leq \kappa \leq 0.60$ indicates moderate agreement; $0.61 \leq \kappa \leq 0.80$ indicates good agreement; and $\kappa \geq 0.81$ indicates very good agreement.

The percentage values arising from the digital analysis of the samples were used to compare the efficiency of smear layer removal between the 5 groups. A Kruskal-Wallis (nonparametric analysis of variance) analysis was undertaken to compare the differences between groups with post hoc Dunn multiple comparison tests.

Finally, to assess the reproducibility of the digital imaging technique, 30 SEM images were randomly selected, coded to ensure blinding, and then reevaluated by the digital imaging technique 3 months later in a fully blinded manner. The weighted kappa statistic was used to compare the 2 evaluations.

Results

The Fleiss' kappa analysis showed good agreement between the 3 evaluators and the digital method ($\kappa = 0.779$) and very good agreement when the scores were 5 (patent dentin tubules) (Table 2). The weighted Cohen's kappa statistic showed very good agreement between each of the 3 evaluators and between the evaluators individually and the digital method.

A Kruskal-Wallis test of the data for digital assessment of all groups showed an extremely significant variation ($P < .0001$). A Dunn multiple comparison test showed that there was a significant difference between the positive control for smear layer (group 5, nickel-titanium [NiTi] + water irrigation) and groups 1–4 ($P < .001$, $P < .01$, $P < .01$, and $P < .05$, respectively). There was, however, no statistically significant difference between the laser groups (2 and 3) or between the laser groups and the conventional treatment group (group 1, NiTi + NaOCl and EDTAC).

Reproducibility of the digital scores on 30 images repeated after 3 months showed very good agreement, with a κ value of 0.973.

Discussion

Although several different systems have been used to score the amount of smear layer remaining after biomechanical preparation, the results of the present investigation indicated that image analysis meth-

ods that express the patency of dentinal tubules might provide a viable and objective alternative to conventional methods with panels of trained observers.

Digital image analysis methodologies can overcome potential evaluator bias and sources of error with panels of evaluators such as fatigue and consistency during long periods. In this study we did note variations between evaluators (Table 2); however, because the images were seen and rated only once by the individual evaluators, we did not have the opportunity to examine intraoperator variability, which might have revealed variations from day to day.

With both conventional and digital approaches, the original SEM images must not be overexposed. Although images that are slightly underexposed can have their gamma corrected so that the image data are usable for analysis, overexposed images have little or no usable data. This is because of the problem of "clipping" (loss of image information) that occurs in overexposed images where the gray scale values approach pure white, and "blown-out highlights" do not contain any detail (22).

The objective nature of digital image analysis makes it better suited to studies in which large numbers of images are to be assessed. Digital methods might be less costly because less time is required for training of evaluators and collection of assessment data. A further advantage of using image analysis is that other parameters of interest can be measured, for example, the density or average diameter of dentin tubules.

The final advantage of the digital approach is that by drawing directly on the images from the frequently cited article of Hulsmann et al. (12), a clear association can be drawn between data gained from the traditional approach and the image analysis approach. This is important for comparing future work with past studies, such as would be undertaken for meta-analyses. The present study showed good to very good agreement between the traditional Hulsmann evaluation technique and the digital imaging method and very good reproducibility. Therefore, this study validates its use for future research (23), for example, for comparing differences between novel methods of smear layer removal.

Conclusion

The use of image analysis techniques to assess the quality of a prepared surface within the root canal system correlates well with the traditional approach, but it offers greater scope for analysis of dentin tubule characteristics. In addition, image analysis can be used to provide additional quantitative data regarding the surface under study.

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