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# SCANNING ELECTRON MICROSCOPIC EVALUATION OF ROOT CANAL IRRIGATION WITH SALINE, SODIUM HYPOCHLORITE, AND CITRIC ACID

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# ABSTRACT

This study used a scanning electron microscope and a calibrated scoring system to quantitatively evaluate the amount of superficial debris and the smeared layer that remained following root canal preparation with six different irrigation regimens. Sodium hypochlorite (NaOC1) was significantly more effective than citric acid in removing superficial debris from the apical third of the treated root canals. However, citric acid or a combination of NaOC1 and citric acid was more effective than either NaOC1 or saline in removing the smeared layer from the surface of the prepared root canal walls.

Thorough debridement of a root canal system is considered essential for long-term success following root canal therapy. However, studies have shown that currently used debridement techniques do not completely cleanse all of the root canal system (1-7). The use of an irrigating solution as an adjunct to instrumentation has long been advocated (8,9). Studies evaluating the effectiveness of various methods have used light microscopy, and more recently electron microscopy. Studies (2,4) which used a light microscope to evaluate the effectiveness of irrigants found superficial hard and soft tissue debris on the canal wall after root canal preparation. Other studies (5-7) which used the scanning electron microscope (SEM) have demonstrated that, in addition to superficial debris, there is a smeared layer covering the canal wall. Some studies (4-7, 10-14) have demonstrated that not all of the canal wall is instrumented during canal preparation and that the smeared layer seems to be found only where endodontic instruments have scraped the surface of the canal wall during canal preparation. The smeared layer is thought to be an amorphous layer that contains primarily fine inorganic particles with some organic material (6-7, 13-16).

The clinical significance of the smeared layer and other superficial debris has yet to be well demonstrated. Although the indications for removal of the smeared layer have been questioned, a number of studies (6,10,14,16-21), have shown that it can be removed.

Unfortunately, past studies (6,10,11,14,16-21) which have evaluated the ability of various irrigation regimens to remove superficial debris and the smeared layer have not been quantitative evaluations. For the most part, studies (6,10,11,14,16-21) have reported either representative samples or a consensus of results.

The purpose of this study was to try to quantitively evaluate the ability of saline (0.9% sodium chloride), 5.25% sodium hypochlorite (NaOCl), and 50% citric acid, used either alone or in various combinations, to remove the superficial debris and the smeared layer during root canal preparation. The prepared canals were examined with a SEM, scored by examiners using a standardized system, and the scores subjected to statistical analysis.

# MATERIALS AND METHODS

Single canal distal roots of 36 extracted human mandibular molars that had been stored in 10% formalin were used in this study. The teeth were randomly divided into six groups. Two endodontists (JCB and CMB) each prepared three root canal specimens in each group. Following a conventional access opening, a #10 K-type file was positioned in the distal root canal until it was just visible at the apical foramen. A working length 1 mm short of this distance was used for root canal preparation. The apical portion of the root was then covered with utility wax to prevent irrigation through the apical foramen, and the teeth were placed in a manikin to simulate patient-treatment conditions. A stepback type of canal

preparation using sequentially sized K-type files was used for canal preparation. The instrumentation time and the quantity of irrigant were both carefully controlled. Beginning with a #10 file, each sequentially sized file was used for 30 seconds followed by a 5-second recapitulation of the canal to the established working length with a smaller file. Files, size #10 through size #25, were used to the predetermined working length. For each succeeding file up to size #50, the working length was shortened by about 1 mm. Following the use of each file above size #25, the root canal was recapitulated for 5 seconds with a size #25 file to the established working length. After completing the coronal access preparation and then after canal preparation with each sequentially sized file, the canal was irrigated with a total of 3 ml. of the irrigants(s) being evaluated and left flooded during subsequent instrumentation. When a combination of two irrigants was evaluated, irrigation with 1.5 ml. of the first solution was followed immediately by 1.5 ml. of the second solution. Irrigation was accomplished using a  $\Im$  ml.syringe(s) that had a 23-gauge notched tip root canal irrigation needle (NPD Dental Systems, Inc., Melville, N.Y.), which was placed into the canal until slight resistance was felt. At completion of canal preparation, the needle would routinely reach to within 1.5-2 mm. of the working length. The quantity of irrigant(s) was carefully controlled so that the same total volume (30 ml.) was used for each regimen.

After completion of the root canal preparation and the final irrigation with the test irrigant(s), the canal was irrigated with 3 ml. of sterile water to terminate any solvent action of the test irrigant(s) and to remove any precipitate that may have formed from the test irrigant(s) themselves (1,10). All canals were then dried with paper points. The canals in Group VI were then given a final recapitulation with a #25 file to the original working length. A reaming action was used in an attempt to remove any loose debris that may have been left in the canal.

The six irrigation regimens evaluated were:

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Group 1	0.9% saline (3 ml.)
Group 2	5.25% NaOC1 (3 m1.)
Group 3	50% citric acid (3 ml.)
Group 4	5.25% NaOCl (1.5 ml) - 50% citric acid (1.5 ml.)
Group 5	50% citric acid (1.5 ml.) - 5.25% NaOCl (1.5 ml.)
Group 6	5.25% NaOC1 (3 ml.) - final recapitulation (#25 file)
	after drying canal.

After the treatment of each tooth was completed, a cotton pellet was placed in the distal orifice and the tooth was removed from the manikin. The distal root was amputated using a heatless disc, longitudinally grooved on the buccal and lingual surface without penetrating the canal, and fractured in half. Both fractured halves of each root were mounted on a single stub and prepared for SEM examination. The specimens were coded by a technician for blind evaluation by three examiners (JCB, DDP, and CLM).

The evaluation of the amount of superficial debris and the extent of smeared layer was done only in the middle and apical third of each root canal. The coronal one-third of the root canals was not evaluated because remnants of the cotton pellet used to block the canal orifice and cutting debris from the root amputation could affect the amount of superficial debris in this area.

It was decided to use a scale of one to four to rank-order the amount of superficial debris and smeared layer in the test specimens. A score of one represented little or no superficial debris or smeared layer, four represented heavy amounts and two and three reflected gradations between the extremes. It was also decided to use a magnification of 75X to evaluate the superficial debris and 800X to evaluate the smeared layer. These magnifications were chosen because they best showed the detail required to make an accurate evaluation while still maintaining as large a field as possible.

Prior to scoring the test specimens, the three examiners reviewed the specimens which had been used in the pilot study. During this time, a general consensus was developed among the examiners as to what amounts of superficial debris and smeared layer constituted a gradation of 1, 2, 3, and 4. In addition, four reference photomicrographs of the superficial debris at 75X (Fig. 1) and four of the smeared layer at 800X (Fig. 2) were taken to represent the four gradations of the scoring system.

These reference photomicrographs were used as standards by the examiners during the subsequent scoring of the test specimens.

Each individual test specimen was scored in the following manner. The half of the root canal that best showed the greatest extent of the apical two-thirds of the root canal was selected for evaluation. First, the apical and middle thirds of the canal were scanned at 75X with all three examiners present. Using the reference photomicrographs for the superficial debris, each examiner simultaneously and independently recorded their superficial debris scores. Next, the apical and middle thirds were re-scanned at 800X and the examiners simultaneously and independently recorded their smeared layer scores using the reference photomicrographs for the smeared layer. The examiners' scores were submitted to statistical analysis to determine if there was any significant differences in the ability of the irrigation regimens to remove either superficial debris or the smeared layer to test the reliability of the scoring system.

#### RESULTS

Table 1 shows the degree of concordance for the scores of the three examiners. All three examiners agreed on the same score 69.4% of the time when evaluating the amount of superficial debris, and 79.2% of the time when evaluating the amount of smeared layer. In all 144 evaluations, at least two out of three examiners agreed on the same score. In only one instance did the score of an examiner differ by more than one unit from the scores of the other examiners.

Table 2 gives the cumulative scores for each irrigation regimen. These scores for superficial debris and smeared layer for the middle and apical thirds were obtained by adding the scores (1-4) given to each of the six specimens by each of the three examiners. Thus, the lowest possible score for any category (i.e. little or no superficial debris or smeared layer) would be 18, and the highest possible score (i.e. the heaviest amount of superficial debris or smeared layer) would be 72.

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Table 2 also gives the Kruskal-Wallis one-way analysis of variance (ANOVA) for the overall differences between the scores of the six irrigation regimens. The Kruskal-Wallis ANOVA was used to test the hypothesis that the distribution of superficial debris and smeared layer scores was not the same for all irrigant groups. For superficial debris, the overall differences were not statistically significant (p<.05) in either the middle or apical third of the canals. However, the overall differences among the irrigation regimens for removing the smeared layer were highly significant (p<0.007) for the middle third, and significant for the apical third of the canals at the p<.062 level.

To establish where the differences occurred, the Wilcoxon ranksum test was used for pairwise comparison of the irrigation regimens (Table 3). The only significant difference (p<.05) for removing superficial debris occurred in the apical third where NaOCl did a better job than citric acid. The pairwise comparisons for removal of the smeared layer show several significant differences in both the middle and apical third. These significant differences (p<.05) always occurred between an irrigation regimen with citric acid and one without citric acid.

#### DISCUSSION

This study evaluated the ability of six irrigation regimens to remove superficial debris and the smeared layer by using an SEM and a calibrated scoring system to quantify the amount of superficial debris and smeared layer remaining after canal preparation. The high degree of concordance (Table 1) among the scores of the three examiners proved the reliability of this calibrated scoring system.

Table 2 gives the cumulative scores for each irrigating regimen and the Kruskal-Wallis test of overall differences among the scores of the six irrigation regimens. While the Kruskal-Wallis ANOVA is less powerful than the parametric ANOVA, it does not require the assumption that the response variable be measured on an interval or ratio scale. Since our measurement scale was ordinal, such an assumption could not be made. The Kruskal-Wallis test is limited to determining if there is a significant difference in the debriding ability among the six irrigation groups.

Because each irrigation group size (n=6) was relatively small, it was difficult to demonstrate statistical significance even with what appears to be appreciable differences among the irrigation regimens evaluated. Despite this, a highly significant difference (p<.007) was obtained for the smeared layer in the middle third of

the canals (Table 2). Future studies should use a larger group size to increase the power of the statistical tests. The reader is also advised to interpret the results of the pairwise comparisons (Table 3) with some caution. In order to make pairwise comparisons between six irrigation regimens, 15 separate tests must be performed (Table 3). This has the effect of increasing the likelihood that the null hypothesis may be rejected when it is true (22).

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Physiologic saline has been recommended as a canal irrigant because it is biologically acceptable (5). Saline's effectiveness as a canal irrigant seems to be based solely on its mechanical flushing action, since it has very little demonstrable demineralizing or tissue solvent activity (11). In this study, the quantity of irrigating solution and the method of delivery were carefully controlled so it would be the same for each irrigation regimen. There was no significant difference at the p<.05 level between saline and the other irrigation techniques in removing superficial debris from the apical and middle thirds of the root canals. This was also true for the removal of the smeared layer from the apical third of the canal. However, the middle third pairwise comparison (Table 3) showed saline to be less effective in removing the smeared layer than the combination of NaOC1-citric acid at the p<.05 and less effective than citric acid or citric acid-NaOC1 at the p<.10.

NaOC1 is probably the most widely used irrigant for root canal preparation (23). Numerous studies (2,4,6,8,9,19) have demonstrated

the ability of NaOC1 to remove loose superficial debris and to dissolve organic debris. In this study, it had the lowest (best) scores for the amount of residual superficial debris found in both the middle and apical thirds of the canals. The pairwise comparisons of the irrigation regimens revealed that NaOC1 was significantly better than citric acid in removing superficial debris from the apical third of the canals.

Citric acid has been recommended as a canal irrigant because of its ability to demineralize and remove the mostly inorganic smeared layer (10,11,17). Although Wayman and others (11) showed that 10%, 25% and 50% solutions of citric acid were all effective in removing calcium when used as a root canal irrigant, its exact mechanism of demineralization is unclear. Neuman and Neuman (24) have pointed out that citrate is a unique ion that can remove calcium from calcified tissues in three ways. First, it is an acid with three carboxylic groups that cause acidic demineralization of calcified tissues. Second, it can form a stable complex with calcium. Third, it can form a more soluble crystal by exchanging with the phosphate of the apatite molecule. The complexing ability (25) of citrate tends to increase as the pH increases. This gives it the ability to dissolve calcium salts at neutral, or even alkaline pH, which were previously considered insoluble except in acids. This property makes citrate an important factor in calcium mobilization at a physiological pH (26). However, for this project a 50% (<sup>WT</sup>/vol)

solution of citric acid with a pH of 1.45 (Fisher Accumet pH Meter, Silver Spring, MD) was used for irrigation. This pH is well below the "critical pH" of 5.5 where tooth substance is expected to undergo demineralization in saliva (26) and probably accounts for its effectiveness as a demineralizing agent when used as a root canal irrigant.

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The demineralization effect of citric acid is apparently very rapid. Pashley and others (27), using dentinal discs, showed that after only 5 seconds, a 6% solution of citric acid removed much of the smeared layer and exposes the orifices of the destinal tubules. After 15 seconds, dentin permeability reached a maximum value, and after 1 minute, most of the smeared layer and debris were removed. The effectiveness of citric acid to remove the smeared layer was also supported by the findings of this study. A highly significant overall difference at the p<.007) level between the scores of the six irrigation techniques occurred for removal of the smeared layer in the middle third of the canals (Table 2) while the apical third was significant at the p<.062 level. It is clear from the data (Table 1) that the difference in removal of the smeared layer is between the three irrigation regimens with citric acid and the three regimens without citric acid. The pairwise comparisons (Table 3) showed that citric acid is significantly better (p<.05) than NaOC1 in removing the smeared layer from both the middle and the apical third of the canals. Citric acid was also significantly better

(p<.02) than NaOCl-recapitulation in the apical third. In the middle third, citric acid was better (p<.08) than NaOCl-recapitulation or saline in removing the smeared layer.

Since a single solution that has the ability to both dissolve organic tissue and demineralize the smeared layer is not available, combinations of irrigants have been recommended (11,17,18,21). In this study, the combination of NaOCl-citric acid or citric acid-NaOCl used after different sized instrument, did seem to maintain the characteristics of each solution. The combinations, irrespective of the other in which they were used, were capable of removing both the organic superficial debris and the inorganic smeared layer. Our results seem to be consistent with some of the results of a study by Yamade and others (21) in which a final flush with a combination of EDTA followed by NaOC1 removed both inorganic and organic debris better than other solutions they tested. They felt that not only was the combination of inorganic and organic solvents helpful, but that the sequence in which they were used during the final flush was also important. This was not found to be significant in our study. In our study, the combination of irrigants in Group 4 (NaOcl-citric acid) and Group 5 (citric acid-NaOC1) were used after each different sized instrument, while Yamada and others (21) used the combination of irrigants only during the final flush. They also used a larger volume of irrigant(s) for the final flush than we did, and these differences in irrigation regimens may have

accounted for the discrepancy in results. Their results with citric acid were inconsistent. In some of their citric acid irrigated specimens, they noted the presence of crystals, which apparently precipitated from the solution. This was avoided in our study by the use of a final irrigation of the root canal with 3 ml. of sterile water.

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After drying the root canal with paper points, a final recapitulation has been recommended to insure patency (28). An attempt was made to determine the extent of the apical plug following each irrigation regimen, but was abandoned because of the unpredictable way the roots split through the apical canal orifice. It was apparent in this study (Table 2) that a final recapitulation increased the amount of superficial debris present in both the middle and apical third of the canals (Tables 2 & 3). Recapitulation of the canals seems to either scatter existing debris from the apical plug or actually create new superficial debris on the canal wall. The difference in the superficial debris scores in the apical third between NaOC1 and NaOCl-recapitulation was significant at (p<.06). The effect of the final recapitulation of the root canal on the smeared layer was difficult to evaluate because this was done only with NaOC1 as the irrigant. However, the amount of smeared layer found after NaOC1 irrigation (Group 2) or after NaOC1 final recapitulation was identical (Table 2). Apparently the final recapitulation had no effect on the smeared layer.

Recent studies (17-21) examining root canal debridement have
demonstrated the presence of superficial debris and a smeared layer
on the canal wall, was well as plags in the orifices of dentinal
tubules. Although there is much speculation, the clinical
significance of these entities is not fully understood. There is
nuch confusion over whether the smeared layer should be left or
removed. Previous studies have demonstrated that the smeared layer
decreases fluid flow (27, 29-30), decreases permeability of
isotopes (27,31) and decreases bacterial penetration (32-34). It
seems possible that the smeared layer may be a barrier and may
contribute to a better seal following root canal therapy. Conversely,
the smeared layer may be deleterious if it prevents irrigants and
medicaments from penetrating into dentinal tubules that may be
narboring organic tissue and microorganisms. In addition, the
meared layer may impede the penetration of sealer and filling
materials into the dentinal tubules. The present study and other
recent studies (16-21) have demonstrated that the smeared layer can
be removed. However, until we have a better understanding of the
consequences of the smeared layer, the basic question of whether
the smeared layer should be removed remains unanswered.

# CONCLUSIONS

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 Examiners can use the SEM to quantitatively score the amount of superficial debris and smeared layer present after root canal preparation.

- 2. There was no significant difference at the p<.05 level in removal of superficial debris from the middle third of root canals among the regimens tested, when the quantity and mode of delivery of the irrigant are carefully controlled.
- NaOC1 was significantly (p<.05) better than citric acid in removing superficial debris from the apical third of the canals.
- 4. Final recapitulation of a canal with an instrument, after completion of canal preparation with NaOCl as the irrigant, increased the amount of superficial debris in both the middle and apical thirds of the canal, and was significant at the p<.06 level in the apical third.
- 5. Saline or NaOCl, used alone, did not remove the smeared layer.
- 6. Citric acid, used either alone or in combination with NaOCl, was generally more effective than either saline or NaOCl in removing the smeared layer.

The opinions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Commercial materials and equipment are identified in this report to specify the investigation procedures. Such identification does not imply recommendation or endorsement, or that the materials and equipment are necessarily the best available for the purpose.

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Degree of concordance among the three examiners during 144 evaluations Table 1.

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	I rrvination	SUP	ERFIC	IAL	DEBRIS	S	MEARE	DLA	Y E R				
		AP	ICAL	IW	DDLE	AP	ICAL	MID	DLE	Total		Total	
	- econique	Agree	Disagree	Agree	Disagree	Agree	Disagree	Agree	Disagree	Agree	8	Disagree	86
	- Saline	5	-	e c	3	9	0	9	0	20	83.3	4	16.7
2.	NaOC1	2ı		4	2	ß	ſ	9	0	20	83.3	4	16.7
э.	Citric acid	m	ო	ĸ	m	ę	ĸ	4	2	13	54.2	Ξ	45.8
4.	NaOCl-citric acid	4	8	4	2	5	-	4	8	11	70.8	7	29.2
5.	Citric acid-NaOCl	4	8	9	0	ß	-	m	m	18	75.0	9	25.0
6.	NaOC1-recapitulation	4	8	S	-	5	~	5.	*	19	79.2	2	20.8
	- TOTAL	25	11	25	11	29	7	28	8	107		37	
	' ~~	69.4	30.6	69.4	30.6	80.6	19.4	77.8	22.2		74.3		25.7
		*Disagr	eed by 2 u	units (1	case of 1	44 cases	()						

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Irrigation regimens with cumulative scores* (total score of six specimens in each group) a of significance shown by Kruskal-Wallis one-way analysis of variance by ranks
Table 2.

Groups	Irrigation technique used after each preparation sequence	Superficial debris middle third of canals	Superficial debris apical third of canals	Smeared layer middle third of canals	Smeared layer apical third of canals
-	0.9% saline (3 ml)	34	41	66	60
2	5.25% NaOCl (3 ml)	30	28	72	70
m	50% citric acid (3 ml)	38	44	39	46
ধ	5.25% NaOCl (1.5 ml) 50% citric acid (1.5 ml)	33	35	36	44
5	50% citric acid (1.5 ml)- 5.25% NaOCI (1.5 ml)	30	32	44	53
ور	5.25% NaOCl (3 ml)- final recapitulation (#25 file)	41	42	68	70
	kw-x <sup>2</sup>	8.24	4.46	15.94	10.52
	d	.144	.4854	.007	.062
* (18 10	west possible score and 72 hi	ghest possible score)			

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# Table 3. Pairwise tests of differences between irrigation regimens using the Wilcoxon rank-sum test\*

Irrigation regimens	Superf deb	icial ris	Sme la	ared yer
Group 1/Group 2	middle third	apical third	middle third	apical third
saline/NaOCl	.68	.17	.68	.58
saline/citric acid	.23	.52	.07	.17
saline/NaOCl-citric acid	.94	.47	.02	.23
saline/citric acid-NaOCl	.63	.33	.07	.69
saline/NaOC1 final recapitulation	.47	.81	.94	.58
NaOC1/citric acid	.13	.04	.02	.02
NaOC1/NaOC1-citric acid	.69	.38	.01	.03
NaOCl/citric acid-NaOCl	.94	.87	.02	.26
NaOC1/NaOC1 final recapitulation	.30	.06	.69	.94
citric acid/NaOCl-citric acid	.23	.11	.63	.75
citric acid/citric acid-NaOCl	.17	.11	1.00	.38
citric acid/NaOC1 final recapitulation	.94	.69	.08	.02
NaOCl-citric acid/citric acid-NaOCl	.63	.63	.58	.52
NaOCl-citric acid/NaOCl final recapitulation	.47	.23	.02	.03
citric acid-NaOC1/NaOC1 final recapitulation	.30	.17	.08	.26

\* The probability that the irrigation regimen pairs have a significant difference in their debridement ability.

FIGURE LEGEND

- Fig 1. Reference photomicrographs showing the various gradations of superficial debris used to rank-order the specimens (original magnification X75). Reference bars = 100 μm. A. Score of 1; B. Score of 2; C. Score of 3; D. Score of 4.
- Fig 2. Reference photomicrographs showing the various gradations of smeared layer used to rank-order the specimens (original magnification X800). Reference bars = 10 µm. A. Score of 1; B. Score of 2; C. Score of 3; D. Score of 4.



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