Original papers

Effect of sodium hypochlorite, isopropyl alcohol and chlorhexidine on the epoxy sealant penetration into the dentinal tubules

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Abstract

Background. The sealer penetration into the dentinal tubules might be beneficial, especially in necrotic endodontic cases, as it provides the obstruction of the contaminated tubules.

Objectives. To determine the effect of 3 final irrigants (sodium hypochlorite (NaOCl), alcohol and chlorhexidine (CHX)) on the penetration of an epoxy sealer into the dentinal tubules.

Materials and methods. The study was carried out on 60 single-canal human teeth with straight roots. The root canals were prepared to the ISO 40/04 size, using the Reciproc® instruments. The teeth were divided into 4 groups (n = 15). The canals in each group were irrigated according to the following scheme: group 1 (control) – 5.25% NaOCl; group 2 – smear layer removal (40% citric acid (CA) and 5.25% NaOCl) and 5.25% NaOCl; group 3 – smear layer removal (as in group 2), and 40% CA, water and 98% isopropyl alcohol; and group 4 – smear layer removal (as in group 2), and 40% CA, water and 2% CHX. The root canals were filled using the vertical condensation technique with gutta-percha and the porphyrin-labeled AH Plus™ sealer. After 3 days, 1-milimeter-thick cross-section slices were cut from the roots at a distance of 2 mm, 5 mm and 8 mm from the apex. The sections were imaged under a confocal microscope and the sealant penetration depth into the dentinal tubules was measured.

Results. The longest resin tags in all parts of the roots were found in group 4 (CHX), and the shortest in group 1 (control). The mean depth of the sealer penetration (in micrometers) was as follows: 21, 22 and 23 (group 1); 201, 231 and 374 (group 2); 170, 232 and 280 (group 3); and 330, 408 and 638 (group 4) in the apical, middle and coronal parts, respectively.

Conclusions. The final irrigation with CHX resulted in the deepest penetration of the epoxy sealer into the tubules. Isopropyl alcohol had the most negative impact on the sealer penetration into the tubules.

Key words: obturation, chlorhexidine, AH Plus™, confocal laser scanning microscopy, dentinal tubules penetration

Background

The primary factors that affect the success of endodontic treatment are the decontamination of the root canal system, and its tight filling and protection against the oral cavity environment. Due to the complexity of the endodontic system, it is impossible to completely eliminate impurities and microorganisms through mechanical preparation only.[1] In addition, during the root canal instrumentation, a smear layer is formed, which closes the dentinal tubules.[2,3] In the case of endodontic system infections, pathogens occur not only within the root canal and on the surface of its walls, but also penetrate deep into the dentinal tubules.[2,3] The intensive irrigation of the root canals and the dentinal tubules before filling is therefore necessary to remove mechanical processing remnants, the leftovers of the smear layer, and also to effectively decontaminate the whole endodontic space.[2–8] After the smear layer removal, the dentinal tubules are opened, which allows antiseptic irrigants to penetrate and decontaminate the dentin more effectively.[2] Additionally, during the gutta-percha condensation, the sealer is inserted into the open dentinal tubules to provide a potential additional barrier, with the sealing and micromechanical anchoring of the material in the canal.[3–9] The depth of the sealer penetration into the dentinal tubules is influenced by the completeness of the smear layer removal, the obturation method, the type and properties of the sealer, and its interaction with the dentin and the residual remnants of the final irrigant used during irrigation.[3–6,8] For the root canal irrigation protocol, a variety of final irrigants can be used, such as distilled water, saline, sodium hypochlorite (NaOCl), alcohol, or chlorhexidine (CHX). These liquids are characterized by different properties, such as hydrophilicity, the contact angle, surface tension, and osmotic pressure.[10] Sodium hypochlorite decomposes into a hyperosmotic sodium chloride solution, the residue of which can theoretically act as an osmotic barrier against the deeper penetration of the sealer.[11] On the other hand, NaOCl has some lubricating properties, as it can reduce torsional stress during the instrumentation of the root canal.[12] Isopropyl alcohol has a low surface tension, dissolves many hydrophobic substances and displaces water, improves the bond strength of the sealer, and thus has a potentially beneficial effect on the penetration of the hydrophobic sealer into the dentinal tubules.[13,14] In contrast, a CHX aqueous solution is a typical wetting agent with a low surface tension.[15] However, its effect on the sealer penetration is not known.

The null hypothesis of this study is the assumption that the use of alcohol as a final irrigant will increase the depth of the epoxy sealer penetration into the dentinal tubules.

Objectives

The aim of the study was to determine the effect of 3 irrigants (NaOCl, alcohol and CHX), on the penetration of the AH Plus™ epoxy sealer into the dentinal tubules.

Materials and methods

Tooth selection and preparation

The study material consisted of 60 extracted human upper incisors with straight roots and single root canals. The teeth were removed for periodontal reasons. Following the extraction, the teeth were stored in a 1% chloramine solution. After cleaning, the roots were cut at the cementoenamel junction, using a diamond-coated, double-sided separator with continuous water-air cooling.

Root canal preparation

The root canals were opened using ISO 10 size C files (VDW, Munich, Germany). The working length was established by obtaining an anatomical foramen and subtracting 1 mm. The canals were prepared using the crown-down technique with the Reciproc® 25 instruments (VDW). A Silver Reciproc micromotor (VDW) was used at the "Reciproc all" settings, according to the manufacturer's guidelines (a reciprocating motion: 120 degrees counterclockwise and 30 degrees clockwise; 300 cycles/min). Then, the canals were widened with the Reciproc 40 instruments (VDW) and calibrated with ISO 40 size K files (VDW). Before preparation, a small amount of a FileCare® lubricant (VDW) was applied to each instrument, and after using each instrument, the root canals were rinsed with 1 mL of 5.25% NaOCl. During the flaring of the coronal and middle parts of the root canals (with the use of the Reciproc 25 instruments), pecking and brushing motions were performed. The apical parts of the root canals were instrumented with the Reciproc instruments (25 and 40) using a pecking motion. All the preparation of the root canals was carried out by a highly trained operator (W.W.).

Dividing into groups and root canal irrigation

Following the mechanical preparation, the roots were randomly divided into 4 groups (n = 15) and the apical foramina were closed with sticky wax to simulate the natural closed system of the root apex and the periapical tissues.

The root canals in group 1 were rinsed only with 5.25% NaOCl. Thus, this was the control group with no smear layer removal. In groups 2, 3 and 4, the smear layer was removed by double rinsing with 40% citric acid (CA) and 5.25% NaOCl. In group 2, 5.25% NaOCl was used in the final irrigating protocol. In groups 3 and 4, after the smear layer removal, NaOCl was reduced by brief irrigation with CA and immediate intensive irrigation with distilled water to prevent dentin demineralization. In group 3, the irrigation protocol was completed with 98% isopropyl alcohol, and in group 4, the irrigation protocol was completed with 2% CHX. A detailed list of the applied irrigation protocols is presented in Table 1.

The irrigating solutions were introduced into the canals in small portions (0.5 mL), using a 0.4 × 19 mm oblique needle inserted into the canals with an up-and-down motion, to a depth of 1 mm shorter than the working length. During the irrigation steps marked with an asterisk in Table 1, passive ultrasound activation was applied for 5 s to each irrigant portion (0.5 mL). The activation was made using an ISO 35 spreader (VDW) connected to the E1 tip of a Smart Piezo scaler (Mectron, Carasco, Italy). Each irrigation–activation cycle lasted 15 s, which means that 2, 4 and 8 irrigation–activation cycles lasted 30 s, 60 s and 120 s, respectively. The spreader was used with an up-and-down motion, having a 2–3 mm shorter reach than the working length.

Table 1. Irrigation protocols (consecutive steps listed in top-down order) in 4 experimental groups

Group	Irrigation protocol
Group 1	5.25% NaOCl: 120 s – 4 mL*
Group 2	40% CA: 30 s – 1 mL* 5.25% NaOCl: 30 s – 1 mL* 40% CA: 30 s – 1 mL* 5.25% NaOCl: 60 s – 2 mL*
Group 3	40% CA: 30 s – 1 mL* 5.25% NaOCl: 30 s – 1 mL* 40% CA: 30 s – 1 mL* 5.25% NaOCl: 60 s – 2 mL* 40% CA: 2 s – 0.1 mL distilled water: 60 s – 2 mL* 98% isopropyl alcohol: 60 s – 2 mL
Group 4	40% CA: 30 s – 1 mL* 5.25% NaOCl: 30 s – 1 mL* 40% CA: 30 s – 1 mL* 5.25% NaOCl: 60 s – 2 mL* 40% CA: 2 s – 0.1 mL distilled water: 60 s – 2 mL* 2% CHX: 60 s – 2 mL

NaOCl – sodium hypochlorite; CA – citric acid; CHX – chlorhexidine;
* passive ultrasound activation.

Root canal obturation

After the irrigation protocols were completed, the root canals were dried with paper points (VDW), and then filled with gutta-percha and the AH Plus sealer (Dentsply Sirona, Bensheim, Germany) by means of the thermoplastic vertical condensation technique, using a SuperEndo™ device (B&L Biotech, Seoul, South Korea). The sealer was modified with the addition of a saturated alcoholic porphyrin solution to obtain a concentration of 0.1%. The excess alcohol was evaporated from the sealer by blow-drying with room temperature air.

Sample preparation and microscopic assessment

After filling the canals, the roots were left for 3 days in a humid environment at 37°C. Then, with an IsoMet® 1000 precision saw (Buehler, Lake Bluff, USA), three 1-millimeter-thick cross-section slices were cut from each root at a distance of 2 mm (apical), 5 mm (middle) and 8 mm (coronal) from the root apex. The samples were coded and analyzed using a Nikon Ti-E inverted microscope with a Nikon A1 confocal system (Nikon, Tokyo, Japan). Magnifications of ×10 and ×20, and wavelengths of 488 nm and 561 nm were used. Each sample was imaged clockwise in 4 quadrants at an imaging thickness of 25 µm. Digital images were acquired by averaging 50 stacks made every 0.5 µm, starting from 10 µm from the sample surface down to 35 µm. The images were acquired at a resolution of 1024 × 1024 pixels, which gave 0.62 µm/pixel. The images were encoded and the resin tag length measurements were made using the ImageJ 1.45s software (National Institutes of Health, Bethesda, USA) in all 4 quadrants, for each section. A series of 32 measurements was made for each image, at fixed fields arranged radially in relation to the root canal axis every 2.8 degrees (Fig. 1). The measurement result for each field was the average value of the length of the resin tags in that field. After the measurements, the software made the export data with 10 average measurements per sample's quadrant, resulting in 40 average values per sample on each level. The summary data was 600 measurements per group on each level (n = 15 x 40 averaged measurements).

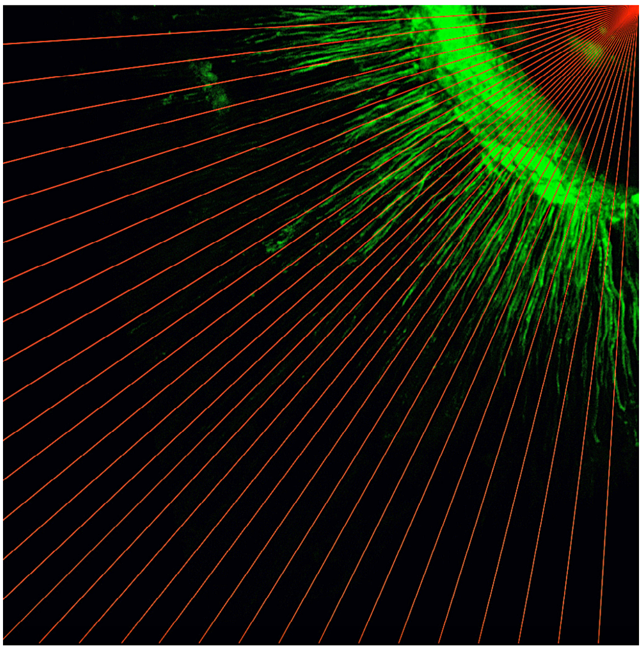

Fig. 1. Photograph of the sample with auxiliary lines. The measurements were carried out at fixed fields arranged radially in relation to the root canal axis

Data collection and statistical analyses

The collected and relevantly encoded data were statistically analyzed using both parametric and nonparametric tests. Since the assumptions for the commonly used in such cases one-way analysis of variance (ANOVA), concluded by Tukey's honestly significant difference (HSD) test at the a posteriori analysis stage turned out to be unfulfilled even after the Box–Cox transformation (the normality Kolmogorov–Smirnov test, Shapiro–Wilk test, Lilliefors test as well as the variance homogeneity tests – Levene's, Hartley's, Fisher's and Bartlett's – failed), the authors decided to perform the analysis using the nonparametric Kruskal–Wallis test followed by relevant nonparametric multiple comparison tests. Although the assumptions for parametric tests were not met, the numerical results were in full accordance with the results of nonparametric tests. This may be assigned to a very large sample size. The obtained results are in line with the additional check based on the reversed decision tree technique. In this case, the usual roles of dependent and independent variables (predictors) are exchanged, namely the irrigation protocol was selected as a dependent variable and the depth of penetration was one of predictors.

Due to the intrinsic characteristic of the data, i.e., the qualitative data ordered on 3 integer levels (the number of the canal sections), the correlation analysis based on Pearson's r parametric test would lead to wrong statements. Thus, for the correlation analysis, Kendall's tau nonparametric test was performed.

In all tests, a significance level of $p \leq 0.05$ was assumed. For the sake of accuracy, the p-values are given with 4 digits following the decimal point.

Results

The values of the depth of the sealers penetration into the dentinal tubules in the studied groups are presented in Table 2. As shown in Table 3 and Table 4, statistically significant differences were found between all pairs of groups ($p < 0.0001$) in the apical and coronal parts of the canals.

Table 2. Depth of the sealer penetration into the dentinal tubules [μm]

Group	Level of 2 mm		Level of 5 mm		Level of 8 mm	
	M ±SD	Me	M ±SD	Me	M ±SD	Me
Group 1	21.0 ±11.2	19	22.0 ±11.6	20	23.0 ±11.9	22
Group 2	201.2 ±35.6	198	230.9 ±64.3	237	374.0 ±87.9	376
Group 3	169.5 ± 52.5	162	231.5 ±66.5	235	280.0 ±103.0	260
Group 4	330.0 ±80.0	325.5	408.0 ±106.5	403	638.0 ±178.0	629.5

M – mean; SD – standard deviation; Me – median.

Table 3. Raw results of the Kruskal–Wallis analysis of variance (ANOVA) rank tests

Section	Number of groups	Number of samples per group	p-value
Apical (2 mm)	4	600	<0.0001*
Middle (5 mm)	4	600	<0.0001*
Coronal (8 mm)	4	600	<0.0001*

* statistically significant.

Table 4. Raw results of the Kruskal–Wallis rank multiple comparison analysis

Section	Group	p-value		
		group 2	group 3	group 4
Apical (2 mm)	group 1	<0.0001*	<0.0001*	<0.0001*
	group 2	–	<0.0001*	<0.0001*
	group 3	–	–	<0.0001*
Middle (5 mm)	group 1	<0.0001*	1.0000	<0.0001*
	group 2	–	<0.0001*	<0.0001*
	group 3	–	–	<0.0001*
Coronal (8 mm)	group 1	<0.0001*	<0.0001*	<0.0001*
	group 2	–	<0.0001*	<0.0001*
	group 3	–	–	<0.0001*

* statistically significant.

In the middle part of the canals, statistically significant differences were found between group 4 and all the other groups ($p < 0.0001$), between group 2 and group 3, and between group 2 and group 1 ($p < 0.0001$ in both cases). However, no statistically significant difference in this section was found between group 1 and group 3 ($p = 1.0000$). For all groups, a statistically significant correlation was found between the root part and the sealer penetration depth ($p < 0.0500$), employing Kendall's tau correlation coefficient (Table 5). However, while the tau correlation coefficient indicates that for groups 1, 2 and 4 there is a positive dependency between the section of the canal (i.e., the distance from the root apex) and the depth of the sealant penetration, for group 3, the depth of penetration does not depend on the part of the canal. This might result from the fact that the sealant penetration in group 3 was the worst, and thus it was equally bad along the whole canal.

Table 5. Kendall's tau correlation coefficients regarding the sealer penetration in relation to the canal section for all experimental groups

Group	Tau correlation coefficient	p-value
Group 1	0.5921	<0.0500*
Group 2	0.0494	<0.0500*
Group 3	0.3995	<0.0500*
Group 4	0.5614	<0.0500*

* statistically significant.

The representative images of the experimental groups are shown in Fig. 2–5.

Discussion

Decontamination and a tight seal on the root system are fundamental to the success of endodontic treatment. During chemical and mechanical preparation, the surface of the canal walls is modified by the activity of chelating agents (demineralization) and NaOCl (deproteination).[2,3] Even after drying the root canals, the open dentinal tubules constitute a potential micro-reservoir for the final irrigant that was used at the end of irrigation.[3,4,6] Thus, the physicochemical properties of the final irrigant can influence the interaction between the sealer and the dentin, and the sealer distribution to the dentinal tubules.

In this study, group 1 served as the control group, since the root canals were irrigated only with NaOCl. The lowest values of the sealer penetration into the dentinal tubules were recorded in this group. Most likely, the smear layer that was not removed acted as a mechanical barrier to the sealer. In the remaining groups, the smear layer was removed by double irrigation with CA and NaOCl, using passive ultrasound activation. A previous study conducted by the authors shows that this protocol is highly effective in removing the smear layer.[16] After removing the smear layer, the canals were irrigated with NaOCl to dissolve the exposed (due to demineralization) protein structures. For the final irrigation in group 2, NaOCl was used; NaOCl spontaneously decomposes into a hyperosmotic sodium chloride solution after a few minutes. Despite the high surface tension and osmotic pressure of the irrigant, a relatively high penetration of the sealer into the dentinal tubules was obtained. This may be related to the abilities of NaOCl to reduce torsional stress and act as a lubricant.[12] In group 3, 98% isopropyl alcohol was used for the final irrigation, and in group 4, 2% chlorhexidine gluconate aqueous solution was used. Alcohols such as ethanol or isopropanol are commonly used in endodontics.[13,14,17] They are very good solvents and can even help remove the propylene glycol-based calcium hydroxide paste dressing from the root canal.[17] Alcohols enable dentin dehydration and have a low surface tension, so theoretically, their use should have a positive effect on the penetration of the sealer into the dentinal tubules. In the present study, a high concentration of isopropyl alcohol was used in order to optimize the dehydration of the dentin, as the authors assumed that dry dentin should be better penetrated by a hydrophobic epoxy sealer. Despite this assumption, the null hypothesis was rejected, as the results of this study showed that using both NaOCl and CHX for the final irrigation provided better effects as compared to alcohol. A study by Nagas et al. demonstrated that leaving the dentin slightly moist rather than dry was more beneficial in terms of the interaction with the sealer.[18]

In this study, the epoxy AH Plus sealer was used, as it is one of the most commonly used sealers. The epoxy AH Plus sealer is characterized by high chemical stability and a relatively good, fluid-proof seal.[5,9] However, the greatest disadvantage of this type of sealer with regard to its interaction with dentin is its hydrophobicity. The dentine is a hydrated tissue, and thus it may have a low affinity to hydrophobic sealers. The authors assumed that dehydration with alcohol would have a beneficial effect on the penetration of the sealer into the dentinal tubules. However, the results of the experiment demonstrated something completely opposite, i.e., the hydrated dentine with residual NaOCl or CHX led to better effects. Perhaps the beneficial results of CHX were due to its surface properties, as CHX performed significantly better when compared to NaOCl. This might be interpreted as the penetration of an epoxy sealer into the dentinal tubules through capillary action.[5] The use of CHX may result in lowering of the surface tension, and thus optimizing the sealer penetration into the tubules. Alcohol may also have adversely affected the sealers penetration by possibly disrupting the binding of the sealer. This is even more likely, as in everyday practice, alcohols are commonly used to remove the excess sealer from the pulp chamber, due to their ability to dissolve the unbound sealer.

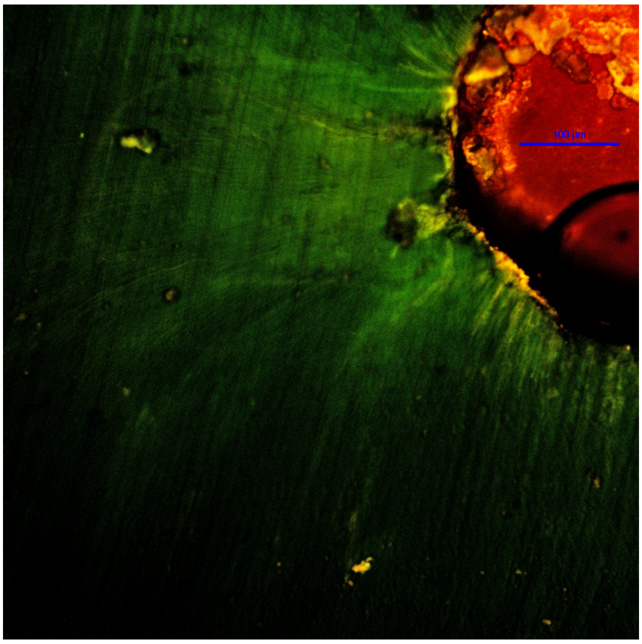

Fig. 2. Representative image for group 1

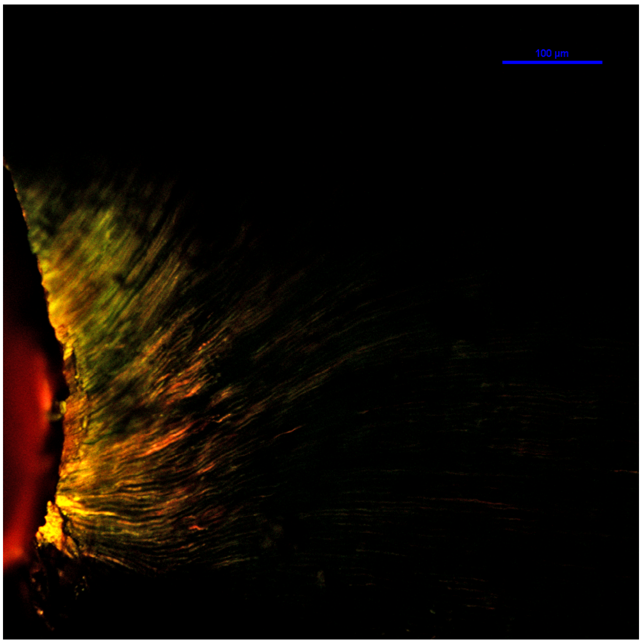

Fig. 3. Representative image for group 2

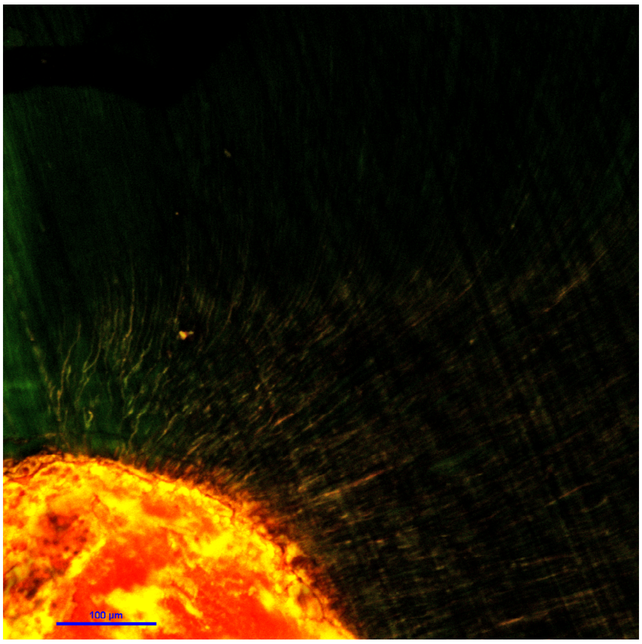

Fig. 4. Representative image for group 3

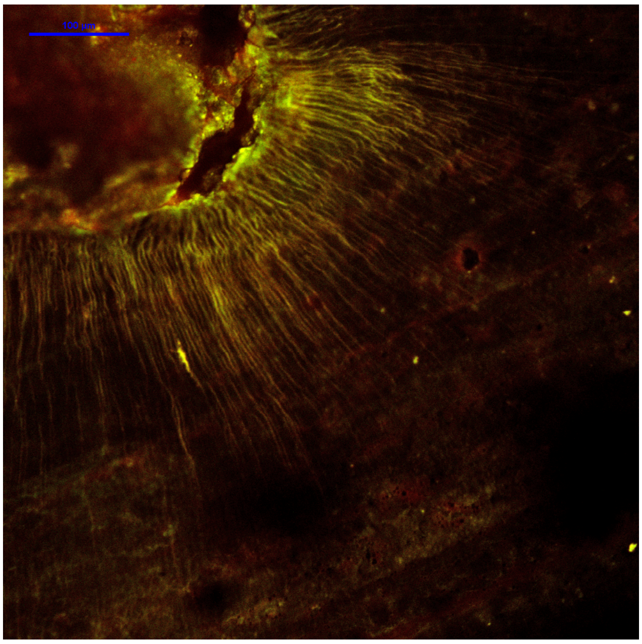

Fig. 5. Representative image for group 4

Limitations

It is important to acknowledge several limitations of this study. Physical properties of the sealer mixed with the marker, such as viscosity and flow, can be different as compared to the normal sealer. Thus, its penetration into the dentinal tubules could in theory be more pronounced in the real clinical scenario. The proper assessment of the sealer penetration may be altered by distribution or sampling errors. The study gives an insight of the tubule penetration only in 3 selected regions, not in the entire space of the root canal. Only straight root canals were selected and used in this study. The penetration of the sealer into the dentinal tubules in curved root canals could be different than in this study. The values of the measured sealer penetration should not be treated as precise data that represent the clinical outcomes. They should be perceived as the reflection of the phenomena in the sealer–dentin interaction after applying different irrigation protocols.

Conclusions

Based on this study and considering its limitations, it can be concluded that:

– the use of CHX as the final irrigant resulted in the deepest penetration of the epoxy sealer into the dentinal tubules;

– isopropyl alcohol had the most negative impact on the sealer penetration into the dentinal tubules;

– the final rinse with NaOCl led to better results as compared to the use of alcohol, but worse as compared to the use of CHX;

– in some kinds of treatment, the sealer penetration may depend on the canal section; the nature of this phenomenon requires future in-depth research.

Further research using different methodologies should be carried out to fully explain the nature of the sealer–dentin interaction.

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