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ORIGINAL RESEARCH

In vitro Assessment of Tooth Color Alteration by Two Different Types of Endodontic Irrigants

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ABSTRACT

Aim: The purpose of this study was to assess *in vitro* the tooth color alterations associated with two commonly used endodontic irrigants, the chlorhexidine gluconate (CHX) and the sodium hypochlorite (NaOCl) up to 15 days post-treatment. Additionally, the possible influence of endodontic access preparation on tooth color was investigated.

Materials and methods: Thirty intact human anterior teeth were used. Black adhesive tape with a 4 mm diameter window was used to standardize the enamel surface intended for color analysis. After the access cavity, preparation and the initial root canal negotiation with stainless steel hand files, the root canal shaping was completed with rotary nickel-titanium files. The teeth were divided into three groups (n = 10). Conventional syringe irrigation was performed with one irrigant for each group. The enamel surfaces were colorimetrically evaluated before access cavity, after cavity preparation and at 1, 3, 7 and 15 days post-treatment. The CIE color parameters (L*, a*, b*) were recorded and averaged for each material and the corresponding color differences (ΔE) were calculated and statistically analyzed.

Results: The most significant factor in tooth color alteration, during the endodontic treatment, was the access preparation. CHX and NaOCl caused tooth color changes comparable with the saline. CHX and NaOCl did not increase the tooth color changes relative to the values induced by the access preparation.

Conclusion: The two endodontic irrigants were not able to induce tooth color alteration to a greater extent than the access preparation.

Clinical significance: Chlorhexidine and NaOCl cannot be considered as discoloring endodontic materials. The most contributing factor in tooth color alteration during endodontic treatment in the anterior teeth is access preparation.

Keywords: Chlorhexidine gluconate, Endodontic irrigants, Discoloration, Sodium hypochlorite.

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INTRODUCTION

Postendodontic tooth discoloration of an anterior tooth is one factor that patients undergo endodontic treatment usually concern.[1,2] The principal causes for the discoloration are decomposition of residual pulp tissue, hemorrhage into the pulp cavity as well as the chemicals and materials used during treatment.[3] Therefore, it is not surprising that the majority of the protocols of endodontic procedure discourage the use of potent discoloring materials.[4]

In order to select essential materials, reliable information about their potential staining properties is required.[3] So far, it is well-known that endodontic agents causing internal staining of dentin are the obturating materials, the intracanal medications and the temporary filling materials.[5] However, due to anatomical complexity of the root canal system, various irrigants are also necessary for an endodontic treatment.[6,7]

The most common irrigant used is NaOCl in a concentration range from 0.5 to 5.25%.[8] NaOCl is a highly effective exhibiting antimicrobial with tissue-dissolving properties.[8,9] CHX has been also recommended for root canal disinfection.[10,11] This is an efficient broad-spectrum, long-lasting antimicrobial agent against a variety of bacteria, especially gram-positive, such as *Enterococcus faecalis*.[12,13]

The staining properties of the endodontic irrigants attracted the research attention the last decade.[14-21] Concerning CHX, clinical studies have recorded promotion of tooth staining when it is used as a mouthrinse for periodontal treatment.[3] The corresponding phenomenon has not been investigated in cases that CHX or NaOCl are used as a single endodontic irrigants. On the opposite, it is already established that the use of CHX and NaOCl in combination during the endodontic treatment causes tooth discoloration.[14,17,18-21]

Therefore, the aim of this study was to assess *in vitro* the tooth color alterations associated with the endodontic irrigants CHX and NaOCl up to fifteen days post-treatment. Additionally, the possible influence of access cavity on tooth color was investigated. The hypothesis was that CHX and NaOCl as single endodontic irrigants do not present differences neither between nor in relation to saline with regard tooth color changes induced within 15 days post-treatment.

MATERIALS AND METHODS

About 30 intact human teeth (8 maxillary central incisors, 4 maxillary canines, 15 mandible central incisors and 3 mandible canines) extracted for periodontal reasons were initially stored in 10% formalin. The teeth were free of caries, restorations and coronal staining. The teeth were cleaned with ultrasonics to disclose gross debris, followed by brushing with a rubber cup and pumice in order to remove remaining debris and stains from the coronal crown surface. Thereafter, the teeth were kept in distilled water until use.

On black rectangular pieces of adhesive tape, a round opening of 4 mm in diameter was cut to match the size of the colorimeter window. The black tape was applied on the facial aspect of the crown in such a way that the round opening was positioned on the cervical third of the crown.

After the preparation of an access cavity of each tooth, the canal was negotiated with a stainless steel size #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) and the working length was determined by subtracting 1 mm of the file length when the tip was visible on the apical foramen. Then, a glide path was confirmed using stainless steel size #15 and #20 K-files (Dentsply Maillefer, Ballaigues, Switzerland) in conjunction with copious amounts of irrigation as described below. Canal shaping was performed with an F2 ProTaper Ni-Ti rotary instrument (Tulsa Dentsply, Tulsa, OK, USA) used in a reciprocating movement under copious irrigation. The F2 was used in conjunction with a 16:1 reduction ratio contra-angle connected to an ATR Vision (ATR, Pistoia, Italy) motor, which allows the reciprocating movement. The CW and the CCW rotations are set on the motor at four tenth and two tenth of a circle. The rotational speed was set at 400 rpm.

The teeth were randomly divided into three groups (n = 10), each according to the irrigant solution used for canal preparation:

1. 2% chlorhexidine gluconate (Ultradent Products, South Jordan, UT, USA) (1.5 ml after each instrument)
2. 2.5% NaOCl (1.5 ml after each instrument)
3. Sterile-saline (1.5 ml after each instrument) used as control.

Irrigation was performed using 5 ml disposable plastic syringes with 27-gauge needle tips (Endo EZ, Ultradent Products, South Jordan, UT, USA) placed passively into the canal without binding.

After a final irrigation (5 ml) with the test irrigants per group, the pulp chamber and the root canals were slightly dried with air and the excess of the irrigant was removed with paper points. Afterwards, a sterile cotton pellet was enclosed in the pulp chamber in order to seal the access opening and all specimens were stored individually in a moist environment at plastic airtight pouches.

The color of the exposed enamel window through the tape hole was evaluated by a colorimeter (Microcolor, Data Station, DrLange, Braiveinstuments, Licge, Belgium) according to CIE Lab system (CIE L*, a*, b*) employing a repeated measurements design (n = 3). In order to establish an accurate positioning system for consistent assessments during color measurements, the specimens were mounted on silicon templates. Tooth color was measured before access cavity (reference color), immediately after cavity preparation (baseline) and at 1st, 3rd, 7th and 15th post-treatment days. The ΔL*, Δa* and Δb* values were calculated by subtracting the baseline value from the values of each time post-treatment. The ΔE values, which were used to measure the color changes, were calculated by the equation ΔE = (ΔL*2 + Δa*2 + Δb*2)1/2 (Bureau Central de la Commision Internationale de I 'Eclairage, 1978). L*, a*, b* and ΔE values before and after access cavity preparation were calculated from the total of the specimens of the three treatment groups (n = 30).

The ΔE values at the four post-treatment times were statistically analyzed by two-way analysis of variance (ANOVA) repeated test with endodontic irrigant and post-treatment time as discriminating variables, following by Scheffé's test ($p < 0.05$). One-way ANOVA repeated test was applied to identify the changes in L*, a*, b* by means of post-treatment time followed by Scheffé's test ($p < 0.05$). Alterations in L*, a*, b* before and after access cavity preparation were statistically analyzed by paired t-test ($p < 0.05$). Statistical analyses were performed using SPSS Statistics 17.0 software (SPSS Inc, Chicago, IL, USA).

RESULTS

The ΔE values measured for the two endodontic irrigants (CHX, NaOCl) and the control (saline) at the four post-treatment times are given in Table 1. The results of two-way ANOVA analysis for the aforementioned ΔE values are detailed in Table 2. The results indicate that neither irrigant type nor post-treatment time or their interaction have significant effect on the ΔE values. Therefore, no post hoc multicomparison statistical test (Scheffé's test) was applied for further analysis.

The L*, a*, b* values per treatment group and post-treatment measurement are given in Table 3. Based on the statistical analysis, color parameters exhibited significant alterations during specific time intervals. More specifically, L* presented changes the 3rd and between 3rd and 7th, and 3rd and15th post-treatment days. As far as it concerns a*, changes were observed at the 1st, 3rd, 7th and 15th post-treatment day. On the other hand, b* demonstrated changes only on 1st post-treatment day.

The color parameters measured before/after cavity access were L* (51.62 ± 8/36.74 ± 12.81), a* (0.67 ± 3.27/0.48 ± 7.33), b* (9.33 ± 4.88/10.36 ± 8.01) and ΔE (17.68 ± 8.21).

The parameter L* revealed statistically significant reduction after cavity preparation whereas a* and b* showed no statistically differences.

DISCUSSION

According to the results of the present investigation, the hypothesis examined was verified. All measurements demonstrated the lack of statistical significant differences in tooth color alterations provided by CHX or NaOCl in relation to saline (control) as well as between the two irrigants, up to 15 days post-treatment.

In terms of CHX as mouthrinse, several possible explanations of its stain promotion have been reported.[3,22] According to the principle mechanism, chlorhexidine acts along with other extrinsic residual factors of the oral cavity in order to promote tooth staining. Such factors are considered the dental pellicle, proteins, carbohydrates, pigmented sulfides of tin and iron produced by denaturation, dietary chromagens and polyphenols. However, in the current study, CHX was used as endodontic irrigant. Thus, the lack of the external contributor factors mentioned above may have limited the staining potential of CHX. In addition, the vital pulp tissue remnants, as possible contributing factor, were also absent since the specimens were extracted teeth. Moreover, since the experiment was extraorally performed, saliva, hemorrhage, tissue fluids, dietary particles and other components (polyphenols) were absent into the root canal system.

As far as NaOCl concerns, tooth color alterations may be induced by its tissue-dissolving and bleaching capacity.[23,24] For example, pulp tissue remnants and residual blood clots are dissolved in excess of NaOCl minimizing the possibility of tooth staining.[25] On the other hand, dentin debris and organic material dissociates NaOCl into Na+ and Cl- ions.[26,27] At the same time, released oxygen-derived free radicals react and oxidize staining molecules like chromophores, resulting in stain removal and bleaching.

In our investigation 2.5% NaOCl solution, at room temperature was used for less than 10 minutes. Under these conditions, NaOCl may exert slight deteriorative effect on the chemical composition of dentin, which cannot induce detectable tooth color alterations. Furthermore, the depth penetration into dentin obtained by 2.5% NaOCl is shorter relative to that caused by 6% NaOCl for 20 minutes at 45°C.[28] As a result, the bleaching capacity of NaOCl was negligible in our specimens.

Generally, values of $\Delta E < 1$ are regarded as not perceivable by the human eye, $\Delta E < 3.3$ as visually perceptible by skilled operators, but considered clinically acceptable, while values of $\Delta E > 3.3$ are considered as clinically not acceptable.

Table 1: ΔE values after the three endodontic treatments (CHX, NaOCl and saline) at four post-treatment times

Irrigant	*Post-treatment time*	*ΔE (mean ± SD)*
CHX	1 day	12.47 ± 8.71
	3 day	14.35 ± 10.10
	7 day	11.32 ± 7.62
	15 day	13.64 ± 9.60
NaOCl	1 day	14.35 ± 9.06
	3 day	15.37 ± 10.82
	7 day	13.11 ± 9.51
	15 day	12.59 ± 8.02
Saline	1 day	15.89 ± 5.37
	3 day	17.76 ± 8.77
	7 day	16.37 ± 6.83
	15 day	15.56 ± 6.55

SD: Standard deviation

Table 2: Two-way analysis of variance results for the ΔE with respect to the three endodontic treatments (CHX, NaOCl and saline) at four post-treatment times

Source of variation	*df*	*Mean square*	*F*	*Sig.*
Corrected model	11	25.426	0.342	0.973
Treatment type	2	93.582	1.257	0.290
Time intervals	3	21.696	0.291	0.831
Treatment type *time	6	5.620	0.075	0.998
Error	81	74.449		

DF: Degrees of Freedom; Sig.: Significance

Table 3: L*, a*, b* values (mean ± SD) for the three endodontic treatments (CHX, NaOCl, saline) at the four post-treatment times

Treatment type	*Post-treatment time*											
	1 day			*3 day*			*7 day*			*15 day*		
	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**
NaOCl	43.35 ± 6.10	7.59 ± 6.05	5.45 ± 4.62	47.73 ± 6.87	4.69 ± 4.04	7.09 ± 3.51	44.16 ± 5.44	6.09 ± 5.18	7.29 ± 3.58	44.15 ± 5.53	4.44 ± 4.13	7.66 ± 3.09
CHX	41.81 ± 7.30	5.01 ± 3.10	5.40 ± 4.30	48.20 ± 9.10	5.92 ± 6.03	6.98 ± 4.47	41.28 ± 8.59	3.89 ± 5.12	6.60 ± 4.40	41.96 ± 6.49	5.26 ± 6.41	6.11 ± 4.82
Saline	44.59 ± 8.36	5.47 ± 2.14	6.08 ± 3.26	53.29 ± 5.70	6.93 ± 4.40	8.58 ± 5.41	40.50 ± 5.97	6.38 ± 4.00	6.80 ± 5.10	41.73 ± 7.62	4.97 ± 2.45	7.23 ± 4.29

SD: Standard deviation

Although significant ΔE changes were not recorded for the three endodontic treatments within the 15-day time observation period, color coordinates L*, a*, b* exhibited several statistically significant differences. These differences were independent from the type of irrigant and the interaction term and the only significant effect was by post-treatment time. Higher lightness was noticed after the 3rd post-treatment day, which decreased between 3rd and 7th, and 3rd and15th post-treatment day. Regarding a*, the specimens exhibited a significant increase during all the time intervals employed in the study. This fact indicates a red shift for the tooth color. As far as the b* parameter concerns, a blue shift for the tooth color was observed after the 1st post-treatment day.

The striking effect of this investigation is the color alteration of the crown observed after access cavity preparation. At least, to our knowledge, there is no evidence-based information regarding the tooth color alteration induced by access preparation during endodontic treatment. The present study detected that tooth structure removal resulted in ΔE value up to 17.68 and in down warding of the color lightness. It should be highlighted that all the color measurements after the application of the irrigants CHX, NaOCl did not identify ΔE values above the value obtained after access preparation.

Although several factors affect tooth color lightness such as, surface texture and morphology, degree of enamel mineralization and thickness, tooth angulation, enamel surface wetting, opacity-translucency of dentin,[29-31] it seems that the majority of the above factors remained unaltered during the interventions in tooth specimens. On the other hand, during cavity preparation, a substantial amount of dental coronal dentin is removed. While enamel is a translucent tissue, permitting transmission and diffusion of the light, the main characteristic of dentin is the opacity and the limited light transmittance. After access preparation it is possible that dentin translucency is increased, while dentin opacity is decreased resulting in a significant reduction in lightness. It must be emphasized that lightness is the most important component of color and color perception. Thus in a clinical situation of an endodontic treatment of an anterior tooth, the endodontist must inform the patient that a slight color alteration may result despite the use of proper root canal sealing materials and the careful cleaning of the access cavity.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn:

1. The most significant factor affecting tooth color alteration during endodontic treatment is the access preparation.
2. The endodontic irrigants CHX and NaOCl caused tooth color changes comparable with a neutral agent, such as saline.
3. CHX and NaOCl did not increase the tooth color changes relative to the values induced by the access preparation.

CLINICAL SIGNIFICANCES

The CHX and NaOCl cannot be considered as discoloring endodontic materials. The most contributing factor in tooth color alteration during endodontic treatment in the anterior teeth is access preparation. Every restorative procedure, following endodontic treatment in anterior teeth, should re-establish tooth color lightness for maximizing the esthetic result.

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