Hydrogen Peroxide Versus Sodium Hypochlorite: All a Matter of pH?

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Hydrogen Peroxide Versus Sodium Hypochlorite: All a Matter of pH?

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ABSTRACT

Introduction: Hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl) solutions are similar in that they contain oxidizing agents with a bleaching effect. NaOCl solutions are stable at a high pH, at which they also exert increased cleansing/proteolysis. On the other hand, H_2O_2 solutions are natively acidic, yet gain bleaching power on organic stains when alkalized. It was investigated whether alkalizing a H_2O_2 solution would also let it dissolve soft tissue or increase its bleaching power on blood-stained dentin. **Methods:** The stability of alkalized H_2O_2 solutions was assessed by iodometric titration. Soft tissue dissolution was investigated on porcine palatal mucosa. The bleaching effect (ΔL^*) after 60 minutes of exposure was monitored in blood-stained human dentin using a calibrated spectrophotometer. To compare similar molarities, 2.5% H_2O_2 solutions were used here, and 5.0% NaOCl was used as the positive control, whereas nonbuffered saline solution served as the negative control. **Results:** Adding alkali (NaOH) to the H_2O_2 solutions rendered them unstable in a dose-dependent manner. A H_2O_2 solution of pH 11.1 was chosen for the main experiments (tissue dissolution and bleaching effect) and compared with a native counterpart (pH = 4.7). Alkalizing the H_2O_2 solution had no discernible effect on its soft tissue dissolution or bleaching power ($P = .75$ compared with the native H_2O_2 solution). The NaOCl solution of similar molar concentration had a considerably ($P < .001$) higher tissue dissolving and bleaching effect under current conditions. **Conclusions:** The proteolytic/bleaching effects of NaOCl solutions are unique and cannot be achieved by altering the pH of peroxide solutions. *(J Endod 2021;47:297–302.)*

KEY WORDS

Bleaching; dentin; hydrogen peroxide; tissue dissolution

SIGNIFICANCE

This research highlights the uniqueness of sodium hypochlorite when it comes to its proteolytic effect, which also results in higher bleaching power in blood-stained dentin compared with hydrogen peroxide, even if the latter was alkalized.

The use of strong oxidizing agents as root canal irrigants has a long tradition in dentistry. Solutions of sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) have been used in different parts of the world to irrigate and disinfect root canals[1,2]. Peroxide-containing pastes are still marketed globally as aids in root canal instrumentation and disinfection[3]. What hypochlorite and peroxide-containing products have in common is that they are bleaching agents and, thus, in theory, have the potential to remove discolorations from teeth during root canal treatment. Both chemicals are used in industrial bleaching procedures[4,5] as well as in different types of sanitizers[6]. Despite their similarity in application, these agents differ in one major aspect—their stability at different pH levels in aqueous solution. NaOCl solutions are stable at high pH and are thus sold in this form[7,8]. On the other hand, peroxide-containing formulations are stable in acidic form and have to be stabilized if the pH is to be elevated[9].

In the context of endodontics, there is one reason why NaOCl solutions have become the main irrigants: their unique ability to dissolve necrotic soft tissue[10,11] and biofilm matrix components[12]. At their native pH, peroxide solutions do not display these cleansing effects, which are highly desirable in endodontics[11,12]. A recent study showed that a NaOCl solution (pH = 12) at a lower concentration (molar) than a H_2O_2 solution (pH = 4.5) dissolved all visible components from human whole blood, whereas the H_2O_2 solution did not. Consequently, the NaOCl had a higher bleaching power in human blood-stained dentin than H_2O_2[13].

It is known from NaOCl solutions that they lose their dissolving efficacy on necrotic soft tissues once their pH is lowered[14] and gain extra power in this context if alkali is added to the solution[7]. These pH effects have not been studied in peroxide solutions at concentrations that are relevant in endodontics. It is

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known that the bleaching power of H_2O_2 on wine and tobacco colors increases with pH[15]. In industrial bleaching, the pH of H_2O_2 solutions is elevated because it is more effective at pH levels above 10. The dissociation of H_2O_2 occurs at pH levels between 10 and 12, and the perhydroxyl anion (HO_2-) becomes the predominant ion above a pH of 11. The perhydroxyl anion is thought to be responsible for this increase in bleaching power and especially stain removal[9,16]. However, it is not known whether an increased pH will also render a H_2O_2 solution proteolytic and, thus, could add an effect that is not seen at native pH levels.

In the present study, we created a 2.5% H_2O_2 solution with a pH >11 and then compared its soft tissue dissolution capacity and bleaching effects on blood-stained dentin to those of a native counterpart at a pH of 4.8 and a NaOCl solution of similar molarity (5%).

MATERIALS AND METHODS

Ethics

The porcine palatal mucosa used here was obtained from the local slaughterhouse (SBZ Schlachtbetriebe, Zurich, Switzerland). Human whole blood was excess material used for blood agar plates in microbiology. The teeth in this study were caries-free third molars extracted for reasons not related to the current work. All patients gave informed written consent that these teeth could be used to perform anonymized *in vitro* tests. None of these human-derived materials could be traced back to their donors. Under local law, the current protocol was exempt from the necessity to obtain individual ethics approval[17].

Solutions and Stability Assessment

The solutions used in the current study were made from concentrated stock solutions of NaOCl (PanReac Applichem, Darmstadt, Germany) and H_2O_2 (Sigma-Aldrich, St Louis, MO). Concentrations were verified by iodometric titration[18]. The pH of these solutions was measured using a calibrated pH electrode (827 pH lab; Deutsche Metrohm, Filderstadt, Germany).

In a first set of experiments, the impact of pH on the available H_2O_2 was assessed over the course of 1 hour using iodometric titration. H_2O_2 concentration and pH were measured immediately after mixing 5.0% H_2O_2 1:1 (wt/wt) with deionized water, 0.1 mol/L NaOH, or 0.5 mol/L NaOH. Measurements were repeated at 15, 30, and 60 minutes. These experiments were performed 4 times on different experimental days, with all 3 solutions being assessed simultaneously at room temperature (25°C).

Based on these results, a fresh 1:1 mixture of 0.5 mol/L NaOH with 5.0% H_2O_2 was used as an alkalized 2.5% H_2O_2 solution for the following experiments. This alkalized H_2O_2 solution had an initial pH of 11.1, whereas the native solution had a pH of 4.7. Nonbuffered physiological saline solution (0.9% NaCl; B Braun Melsungen, Berlin, Germany) and 5.0% NaOCl (pH = 12.2) were used as controls with a minimal and maximal proteolytic effect, respectively. The concentrations of NaOCl and H_2O_2 used here were chosen under the assumption of almost complete dissociation (OCl^- and OOH^-), when a 5% NaOCl ($K_{Diss} = 2.9 \times 10^{-8}$ at 25°C) solution corresponds to a 2.3% H_2O_2 ($K_{Diss} = 2.4 \times 10^{-12}$ at 25°C) solution in molarity. This is the case at a highly alkaline pH.

Soft Tissue Dissolution Assay

A simple yet reliable weight loss assay[19] was used to test the soft tissue dissolution efficacy of test and control solutions. Twenty-four frozen specimens of similar volume and surface area were obtained from porcine palatal mucosa with a round punch (8 mm) applied at the crest of the rugae. Specimens were thawed at room temperature in a humid environment. They were blotted dry and then weighed using a precision balance (AT 261; Mettler Instrumente AG, Nänikon-Uster, Switzerland). The specimens were then immersed without agitation in 10 mL test or control solutions for 20 minutes in cylindrical 40-mL polypropylene containers with a plastic lid (Semadeni, Ostermundigen, Switzerland). Subsequently, specimens were immersed in 0.1 mol/L sodium thiosulfate ($Na_2S_2O_3$) to stop the oxidizing action of H_2O_2 or NaOCl, blotted dry, and weighed again. Tests were repeated 5 times ($n = 6$) on different experimental days. In each run, 1 specimen per group was assessed to avoid bias.

The weight loss was calculated in the percent of the original tissue weight. The pH values of the test and control solutions before and after exposure to the soft tissue were determined using a calibrated pH electrode (827 pH lab, Deutsche Metrohm). These experiments were also performed at room temperature (25°C).

Bleaching of Blood-stained Human Dentin

For these experiments, a recently developed model was used[13]. In brief, dentin cylinders were obtained from the roof of the pulp chamber of human third molars, which had been stored at 5°C in a 0.1% thymol solution. Personnel handling the teeth applied all necessary precautions for infection control. Dentin cylinders of a 6.6-mm diameter and 3.5 mm in height were cut using a low-speed sawing apparatus (IsoMetTM LS; Buehler, Esslingen, Germany) equipped with a diamond-coated wheel saw (Struers, Ballerup, Denmark) for horizontal cuts and a water-cooled diamond core drill (Proxxon, Trier, Germany) for vertical cuts. Dentin specimens were embedded in methacrylate (Paladur; Kulzer, Hanau, Germany) to form a cylinder of an 8.8-mm diameter with the coronal dentin surface exposed. This surface was polished (Tegramin 30, Struers) using silicon carbide grinding paper (Prüfag, Schlieren, Switzerland) up to 4000 grit. Subsequently, a hollow cylinder with a diameter of 5 mm was prepared in the middle of each specimen using a water-cooled diamond core drill so that the dentin had a remaining thickness of 1 mm and the lateral walls of the resulting holding chamber a height of 4 mm. Specimens were soaked in 17% EDTA (Kantonsapotheke, Zurich, Switzerland) for 30 seconds to open the dentinal tubules and remove the smear layer and then washed in deionized water.

To stain the dentin specimens, human whole blood was used containing citrate phosphate dextrose as an anticoagulant and a stabilizer consisting of sodium chloride, adenine, glucose monohydrate, and mannitol[20]. Fifty microliters of blood per specimen were pipetted into the holding chamber. Specimens were centrifuged for 2 minutes at 1200*g* (Z320; Hermle, Gosheim, Germany) in 15-mL Corning tubes (Corning Inc, Corning, NY) with their polished dentin surface (assessment side) facing down. Subsequently, the remaining blood was removed from the holding chamber. The specimens were then stored in 100% humidity for 3 weeks at 37°C in an incubator (NCU-Line IL 23; VWR International, Leuven, Belgium). After that period, a stable discoloration is reached, as has been assessed previously[13].

To irrigate the specimens, 50 μL irrigating solution was pipetted into the holding chamber (Fig. 1*A*). Irrigants were replaced (ie, removed and 50 μL fresh solution applied) every 10 minutes. After a total irrigation time of 60 minutes at room temperature (25°C), the solutions were removed. Spectrophotometric measurements were performed before and after irrigation with test and control solutions. A timer was used so that the irrigation times were exactly the same for all specimens. Experiments were repeated 9 times ($n = 10$) on separate days. Color changes on the polished dentin surfaces (Fig. 1*B*) were assessed using a black/white-calibrated spectrophotometer (CM-2600d; Konica

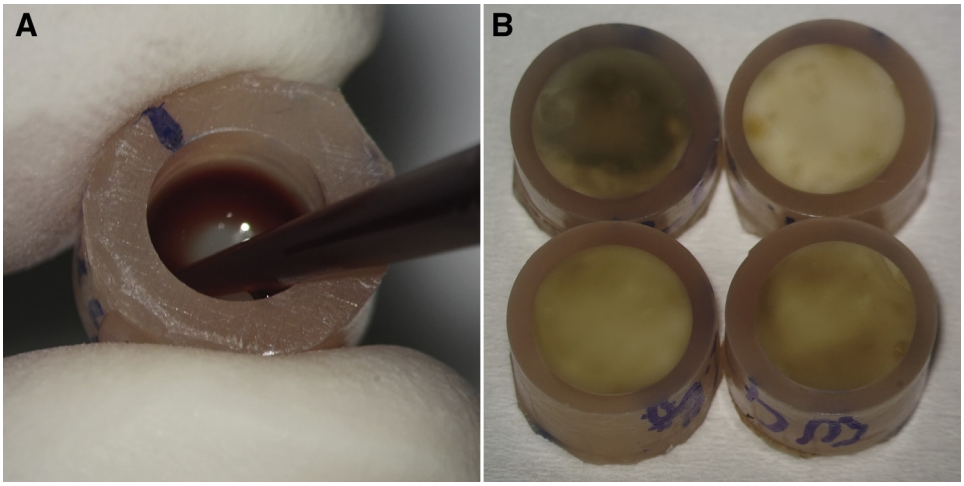

FIGURE 1 – Human dentin specimens that were used as a model of blood-stained dentin. (*A*) Human whole blood was pipetted into the treatment side of the specimens. Subsequently, the blood was centrifuged into the dentinal tubules and left there for 3 weeks at 37°C. These blood-stained dentin specimens were then treated with test and control solutions for 60 minutes, and (*B*) the color change was measured on the assessment side after 60 minutes of treatment with the following solutions: (upper right) nonbuffered saline solution and 5% NaOCl; (lower left) native 2.5% H_2O_2 (pH = 4.7), or (lower right) alkalized H_2O_2 (pH = 11.1).

Minolta, Tokyo, Japan) connected to an external computer running the analysis software (Spectra Magic NX, version 2.8, Konica Minolta). Specimens were positioned on the device using a customized aluminum holder so that they could be reassessed in the same position. Measurements were performed in the CIEL*A*B* color space in the reflectance mode, where the L* value indicates the white to black, a* the green to red, and b* the blue to yellow hue. However, in the current study, only the change in L* values were considered (ΔL*) because this difference represents the bleaching effect[13]. Images were taken with a field of view of 3 mm (Target Mask A147, Konica Minolta) under simulated natural light illumination (D65).

Sample Size and Data Analysis

Changes in H_2O_2 concentration with added alkali over time are presented using descriptive statistics (line graphs representing mean values). All data were distributed evenly, and, consequently, they are presented as means and standard deviations. The exception are pH data, which are logarithmic by nature, and are presented as medians and ranges. The sample sizes for the 2 experiments involving biological samples (soft tissue and blood-stained dentin) were calculated based on the mean values and standard deviations of the first triplicates with a power of 80% and a type I error rate of 5%. The mean values between different irrigants were compared using 1-way analysis of variance followed by the Tukey honestly significant difference test. The alpha-type error was set to 5% ($P < .05$).

RESULTS

There was a dose-dependent negative effect on H_2O_2 stability when alkali was added (Fig. 2). After 60 minutes, the 2.5% H_2O_2 solution with an initial pH of 11.1 had a content of 1.9% ± 0.1% and a pH of 11.3. This solution, which was always created from a fresh mixture of 5.0% native H_2O_2 and 0.5 mol/L NaOH, was used as the alkalized peroxide solution in the subsequent experiments.

The results of the soft tissue dissolution experiments revealed that the alkalized H_2O_2 solution did not gain any discernable proteolytic effect (Table 1). This was contrasted by the distinct soft tissue dissolution efficacy observed with the 5% NaOCl solution ($P < .001$ compared with all other groups). These observations were confirmed in the blood-stained human dentin specimens; no increase in the bleaching effect was observed by the alkalized 2.5% H_2O_2 solution compared with the native (acidic) counterpart ($P = .75$). By far, the strongest bleaching effect was attained by the 5% NaOCl

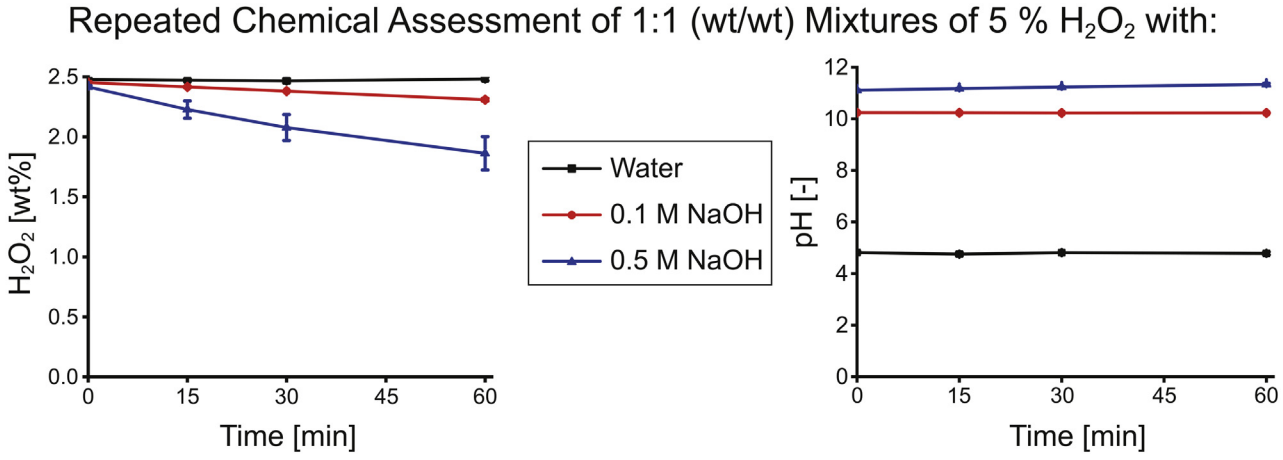

FIGURE 2 – Line graphs depicting the change of H_2O_2 concentration and pH when alkali (NaOH) was added to the solution.

TABLE 1 - The Weight of Standardized Soft Tissue Specimens ($n = 6$) in the Percent of Initial Weight (Means ± Standard Deviations) after 20 Minutes of Immersion in Test and Control Solutions and pH Values (Medians [Ranges]) of these Solutions before and after the Experiment

Solution	Final weight (%)	Initial pH	Final pH
Saline (0.9% NaCl)	102 ± 2^{A}	5.6 (5.6–5.6)	6.6 (6.6–6.6)
2.5% native H_2O_2	100 ± 2^{A}	4.7 (4.7–4.8)	5.7 (5.7–5.8)
2.5% alkalized H_2O_2	104 ± 2^{A}	11.1 (11.1–11.2)	11.2 (11.1–11.2)
5.0% NaOCl	69 ± 8^{B}	12.2 (12.1–12.4)	12.3 (12.2–12.4)

H_2O_2, hydrogen peroxide; NaOCl, sodium hypochlorite.
Different superscript letters indicate that there was a significant difference between data sets pertaining to tissue weight loss at the .001 level (analysis of variance, Tukey honestly significant difference test).

solution (Figs. 1*B* and 3). This difference was statistically highly significant ($P < .001$).

DISCUSSION

This study showed that alkalizing a H_2O_2 solution does not add a proteolytic effect to it, and, therefore, no soft tissue dissolution capacity or increased bleaching effect on blood-stained dentin was observed.

Many things that we know about chemicals with a bleaching effect we know from industrial applications and not in the context of dentistry. For NaOCl solutions, it is known that their proteolytic/cleansing effect is best at extreme pH values (ie, at a very high or very low pH)[21]. However, NaOCl solutions are only stable at a high pH. The opposite is the case for peroxide solutions. They are stable in an acidic environment; yet, their bleaching effect is also increased at a higher pH[9,16]. Both NaOCl and H_2O_2 are oxidizing agents. Based on the reported finding that H_2O_2 solution gains bleaching efficacy against organic stains at a higher pH[15], we speculated that a high pH could also add a proteolytic effect to these solutions, which, under current conditions, could not be confirmed. NaOCl is classified as a halogen-releasing agent, whereas H_2O_2 is a peroxygen[22]. Even though these compounds are among the oldest disinfectants that are still in use, their exact mode of action is not understood completely[9,22]. Interestingly, and perhaps surprisingly, NaOCl, also known as "bleach," has a much stronger bleaching effect on blood-stained dentin than a H_2O_2 solution of comparable concentration. This fact has not received the attention it probably deserves. A bleaching effect on blood-stained roots is a desirable effect, which may be added to the other unique features that make NaOCl indispensable in root canal irrigation[23].

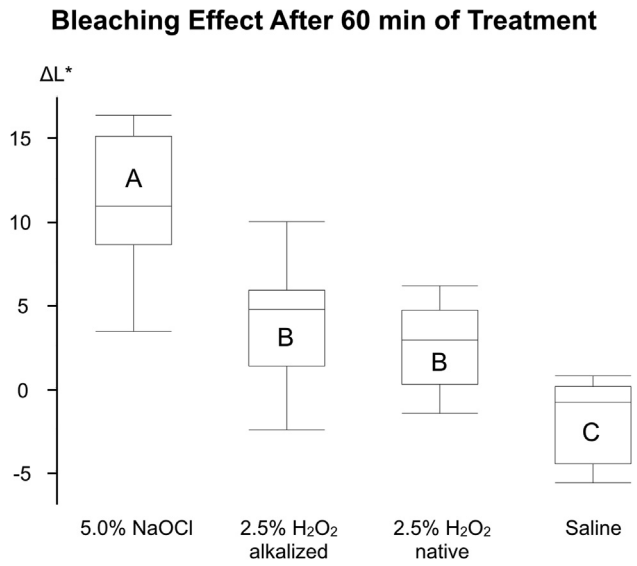

FIGURE 3 – Box plots (medians and interquartile ranges, whiskers: upper and lower data point values) depicting the bleaching effect (ΔL*) of the solutions under investigation. Note that the physiological saline solution that was used as a negative control caused a slight darkening of the specimens, which can be explained by wetting. Data sets that did not differ significantly at the 5% level (1-way analysis of variance, Tukey honestly significant difference test) are marked by identical letters ($n = 10$).

However, the use of hypochlorite-releasing agents (eg, in the form of calcium hypochlorite powder in suspension) as internal bleaching agents would probably not be advisable because a sustained exposure of root dentin to these collagen-depriving agents would affect mechanical dentin properties[24].

As a limitation of this study, it needs to be acknowledged that the experiments were performed at room temperature (25°C) rather than body temperature. Chemical reactions are driven by concentration, pH, and temperature. However, no essential differences in the hierarchy of the outcomes presented here should be expected. This was a laboratory and not a clinical study, and room temperature is laboratory standard.

H_2O_2 was indirectly measured in the current study via a so-called coupled reaction. At first, the available H_2O_2 reacted with an iodide solution to form iodine, which was then titrated with a defined sodium thiosulfate solution. Starch served as the indicator. The consumed amount of thiosulfate solution to reach the colorless end point of the titration indicates the amount of H_2O_2 present. In a simulated clinical approach, Zollinger et al[13] used a higher concentration of H_2O_2 (5%) and a lower concentration of NaOCl (2.5%) than was done here. In the current study, we adjusted the molarities of the solutions under investigation so that the outcomes are more comparable. Because the alkalized peroxide solution under investigation was not completely stable for the duration of the experiments, we deemed 5% NaOCl (which corresponds to 2.3% H_2O_2) and an initial strength of 2.5% H_2O_2 the correct concentrations to tackle the research question under investigation. The results that were obtained are clear-cut; alkalizing H_2O_2 did not possess any desirable effect under current conditions. This could not necessarily have been predicted based on the available literature when H_2O_2 at a higher pH is assumed to be more efficient due to the perhydroxyl anion action. The only article that assessed H_2O_2 pH in the context of bleaching dental tissues showed an increased effect of alkalized solutions against wine and tobacco colors[15]. Two particular differences to the aforementioned study are the use of standard concentrations for root canal treatments, which are lower than concentrations used for bleaching, and the approach to bleach tissue rather than to decolorize mere liquids. H_2O_2 concentrations in this study are also similar to concentrations used in pulp bleaching, but various other factors, such as time, temperature, and material accessibility, are different and not in favor for the system that

was applied here[16]. Bleaching in dentistry with a peroxide system uses rather higher concentrations, as applied in Torres et al[15] and, thus, may compensate for the lack of time, temperature, and other industrial modifications. However, it should be cautioned that the pH of the alkalized H_2O_2 solution used here was still 1 unit under that of the NaOCl solution, and, thus, an extra effect of the alkali contained in the NaOCl cannot be excluded. NaOCl solutions are naturally alkaline because they are produced electrochemically from cooled NaOH (Hooker process)[8]. However, NaOH per se has no effect on soft tissue dissolution[7,25].

The current results suggest that there are systematic differences between the actions of active chlorine and the oxidative species generated by H_2O_2 in an aqueous solution that are not merely a function of pH. How these differences can be explained awaits further clarification. Furthermore, the unique bleaching power of NaOCl solutions should be further assessed in tooth discolorations that are not related to blood/protein.

CONCLUSIONS

This study showed that alkalizing a H_2O_2 solution rendered it slightly unstable; yet, it did not equip it with any discernible dissolving effect on necrotic soft tissue or an increased bleaching power on blood-stained dentin. This study highlights the uniqueness of NaOCl solutions in the context of endodontic treatment needs; their bleaching effect on blood-stained dentin has not gained the attention it probably deserves.

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The authors deny any conflicts of interest related to this study.

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