Evaluation of Hydroxyl Radical Diffusion and Acidified Thiourea as a Scavenger during Intracoronal Bleaching

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Abstract

Introduction: The diffusion of hydroxyl radicals in intracoronal bleaching is associated with a risk of invasive cervical resorption. The use of acidified thiourea has been recommended as a scavenger of residual radicals generated during intracoronal bleaching. The aims of this study were to quantify hydroxyl radical diffusion to external root surfaces after intracoronal bleaching with commonly used materials and to evaluate the effect of using acidified thiourea with hydrogen peroxide (H₂O₂) on hydroxyl radical diffusion. Methods: Eighty-two human premolars were prepared, stained, root filled, and allocated to experimental and control groups as follows: group 1: sodium perborate (SP) and water (n = 21), group 2: H₂O₂ (n = 21), group 3: acidified thiourea and H₂O₂ (n = 21), group 4: neutral thiourea and H₂O₂ (n = 10), control group 1: negative control (water) (n = 10), and control group 2: positive control (SP and H₂O₂) (n = 10). Materials were placed into the pulp chamber, sealed, and placed in 5 mmol/L terephthalic acid at 37°C for 48 hours. Hydroxyl radicals were quantified using a fluorescence microplate reader and high-performance liquid chromatography with fluorescence detection. Results: The H₂O₂ and SP mixture resulted in the greatest hydroxyl radical diffusion and was significantly greater than SP and water (P < .05) and H₂O₂ (P < .05). The addition of acidified thiourea resulted in higher radical diffusion, whereas the addition of neutral thiourea resulted in lower diffusion than H₂O₂ alone. Conclusions: The SP and water mixture resulted in the lowest hydroxyl radical diffusion, and the H₂O₂ and SP mixture resulted in the greatest. Although the addition of acidified thiourea to H₂O₂ did not reduce radicals detected, the addition of neutral thiourea had a positive effect. (J Endod 2016;42:1126–1130)

Key Words
Hydrogen peroxide, hydroxyl radical, intracoronal bleaching, thiourea

Significance
The hydrogen peroxide and sodium perborate mixture resulted in the greatest production and diffusion of hydroxyl radicals during intracoronal bleaching, while the sodium perborate and water mixture resulted in the least and appeared to be the safest bleaching protocol. The use of acidified thiourea with hydrogen peroxide did not reduce hydroxyl radical diffusion to the external tooth surfaces, however, the use of neutral thiourea had a positive effect.

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0099-2399/$ - see front matter
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http://dx.doi.org/10.1016/j.joen.2016.04.010
Although the incidence of bleaching-related ICR is as low as 1.94% (19), it is very destructive and often remains undetected until later stages of the disease process because of inherent difficulties in its diagnosis. Because of this risk, the use of antioxidants and radical scavengers has been recommended to reduce residual hydrogen peroxide and hydroxyl radicals in the pulp chamber (20, 21) and to decrease radicular diffusion of hydroxyl radicals (10).

Thiourea is of particular interest because not only is it a hydroxyl radical scavenger (22); it is also a powerful reductive bleaching agent commonly used in the textile industry (23). An acidified thiourea solution used with 30% hydrogen peroxide in intracoronal bleaching of blood-stained, root-filled teeth was reported to improve bleaching efficacy (24) and was able to reduce hydrogen peroxide and hydroxyl radical diffusion to the external root surfaces (10). However, the latter study used salicylate as a molecular probe, which has been reported to generate multiple reaction products, making accurate quantification of the hydroxyl radicals generated difficult (25, 26). The method of hydroxyl radical detection via the conversion of terephthalic acid into the strongly fluorescent hydroxyterephthalic acid (HTA) is reported to be more accurate in its quantification (27). Therefore, the influence of hydroxyl radical diffusion with the use of acidified thiourea with hydrogen peroxide is worthy of further investigation.

The purposes of the present study were to quantify hydroxyl radical diffusion to external tooth surfaces after the use of various commonly used intracoronial bleaching agents and to evaluate the effect of adding acidified thiourea to hydrogen peroxide in reducing hydroxyl radical diffusion using terephthalic acid as a fluorescent probe.

**Materials and Methods**

**Tooth Sample Preparation**

Eighty-two, noncarious, single-rooted human premolars were collected (ethics approval number: HS-2013-031) and examined using 4.3× magnification (EyeMag Pro; Carl Zeiss, Oberkochen, Germany) under a light source to ensure the cementum layer was intact. The cementoenamel junction was visibly assessed to have no observable damage such as cementum fractures, loss of cementum, or exposed dentin. Following reported protocols (10, 28), the periodontal ligament was removed by gently rubbing with gauze soaked in saline.

Endodontic access cavities were prepared, the working length was assessed. Hydroxyl radical diffusion peaked quickly and reached its maximum within 3 mm below the cementoenamel junction to allow a 3-mm thick Cavit W (3M ESPE, Seefeld, Germany) cervical base to be placed. The Cavit base was extended coronally on the palatal and proximal surfaces to protect the dentinal tubules as recommended in a previous study (30).

### Experimental Groups

The teeth were then randomly allocated into the following experimental and control groups. The experimental groups were as follows: group 1: sodium perborate plus water mixture (n = 21), group 2: 30% hydrogen peroxide (n = 21), group 3: acidified thiourea and 30% hydrogen peroxide (n = 21), and group 4: neutral thiourea and 30% hydrogen peroxide (n = 10). The control groups were as follows: control 1: negative control (water only) (n = 10) and control 2: positive control (sodium perborate and 30% hydrogen peroxide mixture) (n = 10).

The acidified thiourea was made according to a formulation used in a previous study (31). The pH levels of acidified thiourea, hydrogen peroxide, and neutral thiourea were measured with a pH meter (CyberScan pH 10; Eutech Instruments, Thermo Scientific, Waltham, MA) to assess its possible influence on hydroxyl radical diffusion. Bleaching materials were placed into the pulp chamber as described in Table 1. The pH levels of acidified thiourea, hydrogen peroxide, and neutral thiourea were measured with a pH meter (CyberScan pH 10; Eutech Instruments, Thermo Scientific, Waltham, MA) to assess its possible influence on hydroxyl radical diffusion. Bleaching materials were placed into the pulp chamber as described in Table 1. The access cavities were sealed with Cavit (3M ESPE) and Fuji VII (GC, Tokyo, Japan) after placement of the test solution. A pilot study was performed in which the time course of hydroxyl radical diffusion was assessed. Hydroxyl radical diffusion peaked quickly and reached a plateau after 48 hours. Therefore, each tooth was placed in a 5-mmol/L terephthalic acid bath and stored at 37°C in an incubator.

### Table 1. Experimental and Control Groups, Characteristics of Materials, and Method of Usage

<table>
<thead>
<tr>
<th>Groups/Abbreviation</th>
<th>Characteristics and method of usage</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (n = 21)</strong></td>
<td>Sodium perborate and water (SP + water)</td>
<td>SP: EndoPrep; Dentsply, Mount Waverley, Australia</td>
</tr>
<tr>
<td><strong>Group 2 (n = 21)</strong></td>
<td>30% hydrogen peroxide (HP)</td>
<td>HP: ChemLab, Sydney, Australia</td>
</tr>
<tr>
<td><strong>Group 3 (n = 21)</strong></td>
<td>Acidified thiourea and 30% hydrogen peroxide (acid Th + HP)</td>
<td>Th: Chem-Supply Pty Ltd, Port Adelaide, Australia</td>
</tr>
<tr>
<td><strong>Group 4 (n = 10)</strong></td>
<td>Neutral thiourea and 30% hydrogen peroxide (Th + HP)</td>
<td>HP: ChemLab</td>
</tr>
<tr>
<td><strong>Control 1 (n = 10)</strong></td>
<td>Negative control (water)</td>
<td>Laboratory produced</td>
</tr>
<tr>
<td><strong>Control 2 (n = 10)</strong></td>
<td>(Positive control) Sodium perborate and 30% hydrogen peroxide (SP + HP)</td>
<td>SP: EndoPrep, Dentsply</td>
</tr>
</tbody>
</table>

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*(JOE — Volume 42, Number 7, July 2016)*

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chamber for 48 hours before samples of each terephthalic acid bath were tested.

Analytic Method

Hydroxyl radical diffusion was quantified by measuring its reaction product with terephthalic acid, namely HTA, using fluorescence detection with a microplate reader (Synergy Mx Microplate Reader; BioTek, Winooski, Vermont). HTA is a fluorescent molecule with excitation and emission wavelengths of 310 nm and 425 nm, respectively. High-performance liquid chromatography and fluorescence detection (Shimadzu, Secliff, Australia) was used to ensure fluorescence intensity was strictly from the hydroxylation of terephthalic acid by hydroxyl radicals and not by other reaction products. The hydroxyl radicals generated and resultant fluorescence intensity were analyzed statistically using linear regressing of logarithm at a significance level of $P < .05$.

Results

Sodium perborate and water had significantly lower radical diffusion than sodium perborate and hydrogen peroxide ($P < .05$) and acidified thiourea and hydrogen peroxide ($P < .05$). Sodium perborate and hydrogen peroxide had significantly higher diffusion than hydrogen peroxide alone ($P < .05$) (Fig. 1). The use of an acidified thiourea with hydrogen peroxide resulted in higher radical diffusion compared with hydrogen peroxide alone, whereas the addition of neutral thiourea resulted in lower diffusion. Although the differences were not statistically significant, definite trends were observed. This indicates that the pH of a solution may influence radical diffusion, with the lower pH solution causing an increased diffusion and a higher pH solution possibly causing a decreased diffusion (Fig. 2).

Discussion

The ideal protocol for intracoronal bleaching of nonvital teeth is yet to be determined, but it should be aimed at maximizing the efficacy of the bleaching agent while minimizing damage to the adjacent hard and soft tissues. Although hydroxyl radicals are associated with ICR, they are required for the bleaching process to break down stains. Therefore, there must be a balance in which sufficient hydroxyl radicals are generated to achieve effective bleaching results, without causing excessive extraradicular diffusion, which may cause significant damage.

The sodium perborate and hydrogen peroxide combination had the greatest generation and diffusion of hydroxyl radicals through the root surface and crown, and sodium perborate with water had the least. Because there were variable numbers in each group tested, the standard error of the mean was used. The standard error of the mean is calculated from the standard deviation, which also incorporates the sample size of the group in the calculation.

The difference between the 2 groups was statistically significant ($P < .05$). Sodium perborate and water may be the safest bleaching...
agent, and the use of sodium perborate and hydrogen peroxide mixture may be considered with caution.

The acidified thiourea and 30% H₂O₂ group resulted in greater hydroxyl radical detection than the use of 30% H₂O₂ alone. The addition of acidified thiourea did not have a scavenging effect as anticipated, which was in contrast to results of a previous study (10). This may be because of the difference in methodology in hydroxyl radical detection. The current study used terephthalic acid as a hydroxyl radical probe, which has been reported to be highly accurate in its quantification (27).

The increased detection may be explained by the acidified thiourea’s effect on the dentin structure. At a pH of 0.7, the use of the acidified thiourea has a comparable effect as etching the dentin structure with 37% phosphoric acid (pH = 0.8) (32) before intracoronal bleaching, with the exception that the former was not washed away. Etching of dentin opens the dentin tubules and increases dentin permeability (33). Previous studies have shown that acid etching dentin before intracoronal bleaching results in greater diffusion of hydroxyl radicals (34, 35).

A neutral thiourea (pH = 7) solution was used with hydrogen peroxide to test thiourea’s effect on hydroxyl radical diffusion without the confounding influence of the low pH found in the acidified thiourea solution. The addition of neutral thiourea to 30% H₂O₂ resulted in a lower detection of hydroxyl radicals compared with 30% hydrogen peroxide alone and appears to have great potential in reducing the diffusion of hydroxyl radicals. Further studies are required to evaluate the efficacy of bleaching using thiourea, both at acidified and neutral pH values, with 30% hydrogen peroxide.

Although the current study showed the use of acidified thiourea with 30% hydrogen peroxide did not reduce hydroxyl radical diffusion, this combination may be a safer alternative than the commonly used combination of sodium perborate and hydrogen peroxide (Fig. 1). The use of acidified thiourea may offer improved bleaching efficacy because of the possibility of simultaneous oxidative and reductive bleaching (24) and because of the low pH solution allowing greater penetration of bleaching agents (35).

Based on the limitations of this study, sodium perborate with water appears to be the safest bleaching material. It should be the bleaching material of choice in cases involving an immature tooth with wide dentin tubules and in teeth with expected better bleaching outcomes, such as recently discolored and lightly colored stains (36). Teeth of younger patients showed better success rates after intracoronal bleaching (37) because the diffusion through young dentin can be up to twice as high as through older dentin (35). Increased hydroxyl radicals increase the potential damage to fibroblasts, which are the predominant cell type of the periodontal ligament (3); therefore, special care must be taken in bleaching teeth of younger patients to minimize this risk.

In certain situations, it would be desirable to achieve the esthetic result in the minimum number of treatment sessions, and although the combination of sodium perborate and hydrogen peroxide resulted in greater hydroxyl radical penetration, its use may be indicated. Numerous *in vivo* studies showed the combination of hydrogen peroxide and sodium perborate was more effective than sodium perborate and water in decolorizing teeth (6, 38, 39). Ho and Goerig (6) found a 93% success using sodium perborate and hydrogen peroxide compared with 53% success when using sodium perborate and water. The whitening effect of sodium perborate and water can take longer and may require more frequent changes of the bleaching agent (4, 40).

With thiourea’s potential to improve bleaching efficacy through the generation of thiourea dioxide upon reaction with hydrogen peroxide (24), the addition of neutral thiourea in intracoronal bleaching appears to have great potential in improving success and safety. However, further studies are required to determine the optimal pH and concentration of thiourea to use with hydrogen peroxide to maximize the reductive and oxidative bleaching processes while simultaneously offering a radical scavenging effect. Further studies are also required to investigate the bleaching efficacy of neutral thiourea.

In conclusion, the hydrogen peroxide and sodium perborate mixture resulted in the greatest production and diffusion of hydroxyl radicals, whereas sodium perborate and water resulted in the least and maybe the safest bleaching protocol. The sequential use of acidified thiourea with hydrogen peroxide did not reduce hydroxyl radical diffusion, whereas the addition of neutral thiourea resulted in a lower diffusion and is worthy of further investigation.
Acknowledgments

The authors wish to acknowledge Professor Andrew Somogyi and the School of Medicine for use of their research equipment and thank Associate Professor Giampiero Rossi-Fedele for his scientific guidance.

The authors gratefully acknowledge the financial support of the Australian Society of Endodontists.

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

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