

Depletion Rate of Hydrogen Peroxide from Sodium Perborate Bleaching Agent

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Abstract

Introduction: Internal bleaching of discolored teeth uses sodium perborate reacting with water to form the active agent, hydrogen peroxide (H_2O_2). Sodium perborate is replaced at varying time intervals depending on clinician preference and until esthetically acceptable results are achieved, but this is done without scientific basis. This study measured the depletion rate of hydrogen peroxide from sodium perborate as a bleaching agent. **Methods:** Two sodium perborate bleaching products (Odontoblast [Australian Dental Manufacturing, Kenmore Hills, Queensland, Australia] and Endosure Perborate Micro [Dentalife, Ringwood, Victoria, Australia]) and distilled deionized water mixtures at ratios of 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$ were placed into sealed microtubes and incubated at 37°C. H_2O_2 concentrations were measured at 23 time points over 4 weeks. Quantification of H_2O_2 concentrations was obtained using a ferrioxalate oxidation reduction reaction followed by spectrophotometry readings. **Results:** The H_2O_2 concentration rapidly peaked within 27 hours and reached a plateau by about 3 days (75 hours). Low levels of H_2O_2 were evident beyond 3 days and for at least 28 days. No significant differences were found between the 2 sodium perborate products. There was also no significant difference in the depletion rate between the different ratios. **Conclusions:** Based on the chemistry of H_2O_2 depletion, the minimum replacement interval for the bleaching agent is 3 days. Frequent replacements of the perborate clinically may be unnecessary because of the continued presence of low H_2O_2 levels for at least 28 days. Although these data cannot be extrapolated to the clinical situation, they set a baseline for further studies to address the many clinical variables influencing internal bleaching. (*J Endod* 2016; ■:1–5)

Key Words

Hydrogen peroxide depletion, internal bleaching, intracoronary bleaching, sodium perborate, walking bleach

With increasing concerns for esthetics, tooth discoloration is often perceived as undesirable, especially in the anterior region. It is one of the most frequent reasons for seeking dental treatment (1). Discoloration of nonvital teeth after endodontic treatment is relatively common, occurring in 10% of all treated teeth (2) and can result from hemorrhage, incomplete removal of pulp tissues, or the presence of endodontic materials such as root canal irrigants, intracanal medicaments, and filling materials (3). Additional costs related to bleaching can incur a significant financial strain and time burden because of multiple dental appointments (4).

Sodium perborate is a safe, effective, and relatively inexpensive intracoronary bleaching agent used in nonvital teeth in instances in which there is intrinsic discoloration (5). It was first used as a mixture with water (6) and then a mixture with 30% H_2O_2 (7). Sodium perborate has been combined with many carrier agents over time to find the most effective and safe combination. A combination of water and sodium perborate has been found to be safe and equally effective in bleaching compared with mixtures of sodium perborate with 2% chlorhexidine, 37% carbamide peroxide and 30% hydrogen peroxide (H_2O_2), 10% carbamide peroxide, and 35% carbamide peroxide and 35% H_2O_2 (4, 8–11).

H_2O_2 is released from sodium perborate when suspended in water. Its interaction with metal ions, light irradiation, or laser irradiation produces hydroxyl radicals (OH) (12). Many have attributed the shade lightening effect of OH to its ability to break longer-chained chromophores into shorter-chained colorless compounds (13, 14). It has been shown that OH levels increase in an H_2O_2 -dependent manner, confirming that the release of OH is from H_2O_2 (15). Furthermore, adding a chelating agent decreased the release of OH, indicating that metal ions (especially Fe) aid in the production of OH via the Fenton reaction (15, 16).

The most commonly used bleaching technique is the walking bleach method, which involves filling the pulp chamber with a bleaching agent after root canal therapy (17). The cavity is sealed off with a temporary restoration, and the bleaching agent is left in the cavity and replaced regularly until a clinically satisfactory result is achieved (18). However, the replacement time interval has been derived from clinical experience (19), and the optimal renewal time for efficient bleaching has yet to be evaluated. Studies do not have a scientifically justified time period for the renewal of the

Significance

This research is the first to investigate the chemistry of internal bleaching in order to begin to provide a baseline for further research to justify clinical protocols, which to date are based mainly on clinical experience and opinions.

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sodium perborate bleaching agent. The intervals range from 3 to 4 days (20), 3 to 7 days (21), 2 to 6 weeks (22), 12 days (23), 4 to 7 days (24), and 21 days (25).

This study aimed to investigate the chemical reaction of sodium perborate and water releasing H_2O_2 , and its rate of depletion. Because the trend in the rate of release of H_2O_2 from a sodium perborate bleaching agent has yet to be investigated, the results will serve as a scientific foundation for further and more clinically relevant research in the field of intracoronary bleaching. The hypotheses tested were

1. That there were no significant differences in the depletion rate of H_2O_2 between 2 sodium perborate products and
2. There was also no significant difference between the different bleach/water ratios.

Materials and Methods

Preparation

Two brands of sodium perborate powders were used: Odonto-bleach (ODB) (Australian Dental Manufacturing, Kenmore Hills, Queensland, Australia) and Dentalife Endosure Perborate Micro (END) (Endosure Perborate Micro; Dentalife, Ringwood, Victoria, Australia). Initially, the intention was to include 2 g/mL as recommended for clinical use of sodium perborate (26), and a pilot study was developed to determine if this was feasible. Sodium perborate samples (ODB and END) were used at 1, 2, and 3 g/mL Milli-Q water (Millipore Corporation, Molshiem, France) for 16 time points over 6 weeks: 1, 3, 6, 9, and 24 hours and 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, and 42 days. At each time point, negative controls with Milli-Q water and a standard curve consisting of serial dilutions of H_2O_2 were prepared. However, sodium perborate was found to be relatively insoluble and formed a cloudy suspension. Spectrophotometer results were skewed because of this opacity of samples. Furthermore, there was insufficient supernatant available in the 3-g/mL samples for triplicates. The revised methodology reduced the sodium perborate samples to 25, 50, and 100 $\mu\text{g/mL}$ Milli-Q water. This produced samples with minimal to no opacity and an adequate volume of supernatant for analysis.

The revised method involved measurements of H_2O_2 at 23 time points over 4 weeks: 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes; 24, 27, 48, 51, and 75 hours; and 9, 10, 14, 21, and 28 days. Sodium perborate powders (END and ODB) were weighed into 2.2-mL microtubes (Greiner Bio-one GmbH, Frickenhausen, Germany), and each tube contained 50, 100, or 200 μg per product. Milli-Q water (2 mL) was added to every reaction tube so that for each time point, the concentration of the samples were as follows: END 25 $\mu\text{g/mL}$ (END25), END 50 $\mu\text{g/mL}$ (END50), END 100 $\mu\text{g/mL}$ (END100), ODB 25 $\mu\text{g/mL}$ (ODB25), ODB 50 $\mu\text{g/mL}$ (ODB50), and ODB 100 $\mu\text{g/mL}$ (ODB100). All samples were incubated at 37°C.

Measuring the Concentration of H_2O_2 Released

At each time point, 6 samples were removed from the incubator and vortexed for 5 seconds. Triplicates of 100- μL samples were pipetted into a microplate as labeled in Figure 1. At each time point, a fresh set of H_2O_2 standards was prepared with serial dilution to establish the standard curve, and the concentration varied from 10^{-1} mol/L to 10^{-9} mol/L. Triplicates of each concentration were pipetted into the wells on the same plate. Milli-Q water was used as the negative control.

The measurement of H_2O_2 was performed according to the method described by Camps et al (27), and 0.2 mL 10 mmol/L FeSO_4 and 0.1 mL 2.5 mmol/L potassium thiocyanate were added to experimental wells. The microplate was read with a spectrophotometry machine (Wallac VICTOR3 1420 Multilabel Counter; PerkinElmer, Waltham, MA) at a wavelength 480 nm. Results were exported into Microsoft Excel for Mac 2011 Version 12.4.1 (Microsoft Corporation, Redmond, WA).

Analysis consisted of creating a standard curve using the absorbance of the standards, and concentrations of H_2O_2 were determined using the following linear equation: $y = mx + c$, where y equals the average absorbance of each sample type minus the negative (28). The results were graphed and statistically evaluated with 2-way analysis of variance (SPSS version 11.5.0; SPSS Inc, Chicago, IL) with $P < .05$.

Results

The first 27 hours showed a rapid increase in the concentration of H_2O_2 , produced, which peaked at 27 hours before beginning to decline

	25ug/mL			50ug/mL			100ug/mL			-ve control		
END												
ODB												
Hydrogen peroxide [c]												
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}			

Figure 1. The layout of the 96-well plate for 1 time point.

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