Effect of Peroxide Concentration and Brushing on Whitening Clinical Response

Abstract: This clinical trial compared the effects of hydrogen peroxide concentration and toothbrushing on clinical response to vital bleaching. Tooth bleaching was accomplished with a flexible, polyethylene strip coated with a hydrogen peroxide bleaching gel worn for 30 minutes twice daily over a 14-day period. A total of 36 subjects were randomized to 1 of 3 treatment groups: 5.3% hydrogen peroxide strip plus prebrushing, 6.5% hydrogen peroxide strip plus prebrushing, or 6.5% hydrogen peroxide strip without prebrushing. Two groups brushed with regular anticavity toothpaste immediately before bleaching, while the other group performed ad libitum brushing only. Tooth color was measured over a 14-day period using digital images of the anterior dentition. Over the 14-day treatment period, all 3 strip groups experienced highly significant ($P < 0.001$) whitening as evidenced by decreased yellowness ($\Delta b^*$) and increased brightness ($\Delta L^*$), as well as composite color change ($\Delta E^*$) relative to baseline. Keeping brushing constant, the 6.5% hydrogen peroxide strip plus prebrushing group experienced a 31% to 60% improvement in whitening relative to the 5.3% hydrogen peroxide standard. Keeping concentration constant at 6.5% hydrogen peroxide, the prebrushing group experienced a directional 5% to 33% improvement in whitening relative to no prebrushing. All treatments were generally well tolerated. This study demonstrates that for strip-based delivery, increasing hydrogen peroxide concentration to 6.5% results in a significant improvement in efficacy with few tolerability trade-offs.

There is a long-standing history of the use of peroxides in dentistry for various oral conditions.1 Over the past decade, these agents have gained considerable popularity with respect to applications for vital tooth bleaching, partly as a result of the advent of custom bleaching trays.2 Such professionally dispensed systems led to marked improvements in treatment consistency and convenience. Subsequent research established the safety and efficacy of vital bleaching, and helped expand treatment indications.3,4

Vital bleaching can be accomplished using a variety of methods or systems, which can be generally categorized as professionally administered (in-office care), professionally dispensed (as with the popular at-home tray systems), or self-administered (using the various direct-to-consumer bleaching products). While there is a wide variation between methods in terms of formulation, delivery, and regimen, all three methods generally rely on a common peroxide-based chemistry to effect color change. Provided there is sufficient peroxide diffusion, such systems will improve tooth color to some degree. Some reviewers suggest a high degree of similarity in whitening efficacy across many systems.5

As for efficacy, peroxide-based vital bleaching with any of the methods carries risk for two relatively common adverse events—tooth sensitivity and gingival irritation. These events may affect up to two thirds of individuals during the period of active bleaching.6 The tooth sensitivity is described as minor and self-limiting, and often in response to cold stimuli.7 The etiology is believed to be
related to pulpal penetration of peroxide, though other factors, including dehydration or tray-related tooth movement, may account for the observed response. Some suggest that professionally administered, in-office treatment may contribute to increased tooth sensitivity, though this may be technique-specific.8 While a number of products have been developed to deal with tooth sensitivity, including the incorporation of fluoride and potassium nitrate in whitening gels, such products generally lack robust supportive clinical evidence. Gingival irritation is less well described, despite the fact that it is reported to represent the second most common adverse response to bleaching.4 Etiology is unknown, though the use of non-scallapped trays, peroxide contact with tissue, and other factors are believed to contribute to this irritation. The clinical presentation is generally not reported or described with respect to location, duration, or management. Despite the numerous clinical case studies on tooth whitening, there are surprisingly few clinical photographs illustrating oral irritation. Other factors, including attack rates and interventions, are generally poorly described, perhaps because of the rather common observation of generally improved gingival health during bleaching treatment.9-11

Because peroxide is a natural metabolic product, several enzyme systems in saliva and elsewhere limit toxicity.3 These enzymes likely play an important role in both the efficacy and tolerability of vital bleaching. Peroxidase, readily available on tooth and tissue surfaces, affects peroxide degradation.12 This degradation limits peroxide diffusion into hard and soft tissues. Dentifrices contain detergents, such as sodium lauryl sulfate, that may readily denature proteins on contact. Theoretically, toothbrushing immediately before bleaching may have the countervailing effects of improving effectiveness, while contributing to mild, localized, and transient oral irritation.

Vital bleaching with 5.3% hydrogen peroxide whitening strips is reported to be effective and well tolerated with 14 hours of total administration time.13 When measured objectively, strip-bleaching effectiveness is comparable to that achieved with certain 10% carbamide peroxide tray systems at half the contact time of trays. Both the strip and tray systems contributed to minor oral irritation sometime during the 14-day bleaching regimen. Increasing peroxide concentration in bleaching gels should promote faster bleaching, with the possible trade-off of increased tooth sensitivity and oral irritation from increased diffusion. While this effect is established for tray-based systems, a similar response for strip systems has not been demonstrated.

This new clinical study was conducted to evaluate the effects of concentration and before-treatment toothbrushing on the clinical response to whitening strips. The hypothesis was that increased peroxide concentration on the strip would promote whitening, but reduce tolerability. Because before-treatment toothbrushing likely impacts on the enzymatic degradation of peroxide, elimination of this standard pretreatment step should improve tolerability, but reduce whitening. We tested these relationships in a randomized study involving three treatment groups.

### Methods

A randomized and blinded clinical trial was conducted to evaluate the impact of peroxide

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### Table 1—Treatment Groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Bleaching Treatment</th>
<th>Duration</th>
<th>Brushing Treatment</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3% hydrogen peroxide plus prebrushing</td>
<td>5.3% hydrogen peroxide gel strip</td>
<td>30 minutes 2x daily for 14 days</td>
<td>Anticavity</td>
<td>Prebrushing</td>
</tr>
<tr>
<td>6.5% hydrogen peroxide plus prebrushing</td>
<td>6.5% hydrogen peroxide gel strip</td>
<td>30 minutes 2x daily for 14 days</td>
<td>Anticavity</td>
<td>Prebrushing</td>
</tr>
<tr>
<td>6.5% hydrogen peroxide, no prebrushing</td>
<td>6.5% hydrogen peroxide gel strip</td>
<td>30 minutes 2x daily for 14 days</td>
<td>Anticavity</td>
<td>No prebrushing</td>
</tr>
</tbody>
</table>
concentration and toothbrushing on clinical response with strip-based bleaching. The study population consisted of generally healthy adult volunteers who desired to have their teeth whitened. Individuals were excluded if they had preexisting tooth sensitivity or facial anterior restorations, but not because of entry tooth color. After obtaining informed consent, eligible subjects were randomized to one of three treatment groups, balancing for gender (Table 1). In all 3 groups, the whitening strips were applied to the maxillary and mandibular arches twice daily over a 14-day period. In addition, all 3 groups were assigned to a regular dentifrice (Crest Cavity Protection Regular Paste) and a soft profile toothbrush (Crest Complete, extra soft). While all 3 groups used the same dentifrice and brush, 2 groups were instructed to brush immediately before strip application, while the third group was instructed not to brush before bleaching. In this design, the 5.3% hydrogen peroxide wt/wt system and prebleaching brushing represented the positive experimental control.

To ensure blinding, test strips were identical with respect to appearance and labeling. Test products and brushing instructions were packaged in individual test kits, each labeled with a unique subject identification number. Subjects were instructed not to discuss the brushing regimen with study personnel during the course of the trial.

Because groups differed as to the brushing regimen (prebleaching brushing or not), efficacy was measured using digital imaging analysis, an objective and quantitative method for color measurement used successfully in partially blinded clinical trials to demonstrate a peroxide-dose relationship. Digital images of the maxillary and mandibular anterior teeth were collected at baseline, day 4, day 7, and day 14 of treatment. At each time point, subjects were positioned in a chin rest and aligned; then, the facial anterior tooth surfaces were imaged under standard polarized lighting conditions using a high-resolution digital camera (Fuji HC1000 CCD). Red-green-blue (RGB) values were determined for each tooth pixel in the image with reference to a calibration standard, and these values were averaged over all 12 teeth. RGB values were then transformed to derive numerical values for tooth color in terms of L*, a*, and b*, which represent three-dimensional tooth color from light-to-dark, green-to-red, and blue-to-yellow, respectively. Color change (ΔL*, Δa*, and Δb*) was determined by comparing each post-treatment visit to baseline. Negative Δb* (reduction in yellow) and positive ΔL* (increased brightness) were indicative of a whitening benefit. Using a standard method, a composite color change (ΔE*) was derived as the square root of the sum of the squares of the individual color changes. Between-group comparisons for Δb*, ΔL*, and ΔE* were made using analysis of covariance, with the baseline value as the covariate. The between-group treatment comparisons for concentration effects and brushing effects were one-sided.

**Results**

Thirty-six subjects were randomized; 32

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**Table 2—Demographic and Behavioral Characteristics by Treatment Group**

<table>
<thead>
<tr>
<th></th>
<th>5.3% Hydrogen Peroxide Plus Prebrushing</th>
<th>6.5% Hydrogen Peroxide Plus Prebrushing</th>
<th>6.5% Hydrogen Peroxide, No Prebrushing</th>
<th>Two-sided P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Age (SD)</td>
<td>33.3 (5.23)</td>
<td>36.5 (8.57)</td>
<td>39.1 (9.22)</td>
<td>0.22</td>
</tr>
<tr>
<td>Female (%)</td>
<td>11 (92%)</td>
<td>10 (83%)</td>
<td>10 (83%)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Behavioral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee/Tea Use</td>
<td>8 (67%)</td>
<td>6 (50%)</td>
<td>8 (67%)</td>
<td>0.76</td>
</tr>
<tr>
<td>Tobacco Use</td>
<td>2 (17%)</td>
<td>0 (0%)</td>
<td>1 (8%)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

SD = standard deviation
completed all study visits. The study population was predominantly female (86%), and ranged from 24 to 56 years of age. While less than 10% of subjects were tobacco users, most reported daily use of cola, tea, or coffee. Treatment groups were generally well balanced with respect to both demographic and behavioral parameters (Table 2).

Over the 14-day treatment period, all 3 strip groups experienced highly significant ($P < 0.001$) whitening as evidenced by decreased yellow ($\Delta b^*$) and increased brightness ($\Delta L^*$), as well as composite color change ($\Delta E^*$) relative to baseline. These outcomes were evident beginning at day 4, with the average benefit generally increasing incrementally through day 14 (Figures 1 through 3) for each of the color parameters of interest in this study.

Observed whitening was greatest in the two higher-concentration groups relative to the lower-concentration positive control (twice-daily prebrushing plus 5.3% hydrogen peroxide strip treatment). Keeping brushing constant, the 6.5% hydrogen peroxide strip plus prebrushing group experienced superior ($P < 0.02$) overall whitening relative to the 5.3% hydrogen peroxide strip plus prebrushing group (Table 3). This represented 31% to 60% improvements in $\Delta b^*$, $\Delta L^*$, and $\Delta E^*$ in the higher-concentration strip relative to the 5.3% hydrogen peroxide standard. Keeping hydrogen peroxide concentration constant at 6.5%, the prebrushing group experienced a directional improvement in whitening relative to the group without prebrushing. At the 6.5% hydrogen peroxide concentration, prebrushing contributed to an observed 5% to 33% improvement in $\Delta b^*$, $\Delta L^*$, and $\Delta E^*$ relative to no prebrushing. This effect was most evident for $\Delta L^*$, where prebrushing contributed to an observed 0.3-unit improvement in brightness at the end of treatment. However, these two higher-concentration groups did not differ statistically at this time point, or for any other parameter or time point measured in this trial.

All three treatments were generally well tolerated, with tooth sensitivity and oral irritation representing the most common adverse events (Table 4). A total of 7 subjects (19% of the study population) reported tooth sensitivity at some time during the trial. These cases were similarly distributed across all 3 groups, and none of the tooth sensitivity contributed to either early withdrawal or change in the treatment regimen.
There were 13 subjects (36% of the study population) who reported minor oral irritation. Most (85%) of these cases involved individuals assigned to one of the 6.5% hydrogen peroxide strip groups, of which the 6.5% hydrogen peroxide strip plus prebrushing group contributed 6 of the 13 oral irritation reports. One individual, a 47-year-old woman assigned to the 6.5% hydrogen peroxide strip plus prebrushing group, discontinued treatment at day 7 because of mild, persistent oral irritation.

**Discussion**

All three of the strip/brushing treatments in this clinical study were efficacious. Not unexpectedly, increased concentration contributed to increased whitening. In this trial, the 6.5% hydrogen peroxide strip was shown to provide superior efficacy over a marketed, lower-concentration hydrogen peroxide strip. After 14 days of use, this represented a 31% improvement in whitening ($\Delta b^*$) compared to the 5.3% hydrogen peroxide strip used for the same duration.

While the relationship between concentration and response is widely accepted and reasonably well established in the literature, at least for in vitro bleaching, several aspects of this study may be particularly relevant to the in vivo evaluation of the effects of concentration on clinical response. Total dose dispensed and dose distribution represent potential variables.
associated with the tray-based systems that may impact on study outcomes. In contrast, whitening strips carry a uniformly distributed dose, thereby eliminating dosing variability as one of the study parameters. The whitening strips used in this study varied only in peroxide concentration. There were no desensitizing or other agents added at a higher concentration, which in turn introduce other variables into the research. Blinded testing was assured because the test strips were uniform in appearance and labeling, and outcomes were measured objectively using digital image analysis. Finally, the trial included a positive control, the 5.3% hydrogen peroxide strip with prebrushing. The adjusted mean response for this control was $-1.65 \Delta b^*$ after 2 weeks of treatment, within 3% of the response previously reported for this strip concentration and regimen, thereby supporting the validity of the clinical experiment.13

As with efficacy, peroxide concentration is reported to affect bleaching tolerability. Several marketed, higher-concentration products include fluoride or potassium nitrate, ostensibly to reduce tooth sensitivity. In this clinical trial, toothbrushing followed by strip application led to more reported tooth sensitivity and oral irritation for the higher-concentration strip compared with its control, suggestive of a direct relationship between concentration and tolerability. For the higher-concentration strips, tolerability was observed to improve in the absence of immediate prebrushing.

Overall study results suggest that toothbrushing immediately before bleaching may have a modest positive impact on efficacy, while negatively impacting on tolerability. This is consistent with dentifrices playing a minor role in bleaching effectiveness and tolerability, and with previous research on the lower-concentration 5.3% hydrogen peroxide strip.17 Because the magnitude of the treatment effect was observed to be approximately 5% (about a single day’s bleaching), eliminating toothbrushing immediately before bleaching may represent a reasonable trade-off for certain patients.

Both the 5.3% and 6.5% hydrogen peroxide whitening strips were effective when used twice daily over a 14-day period. Increasing hydrogen peroxide concentration to 6.5% resulted in significant incremental whitening relative to the control. Some patients may experience minor tooth sensitivity or oral irritation during bleaching, especially at higher concentrations. In those circumstances, patients may be advised to avoid toothbrushing immediately before bleaching.

References