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Laboratory Research

Tooth Color Change and Erosion: Hydrogen Peroxide Versus Non-peroxide Whitening Strips

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Clinical Relevance

There are uncertainties about non-peroxide strip whitening efficacy and effect on enamel erosion. This study indicates that peroxide whitening strips have superior whitening efficacy compared to non-peroxide strips. None of the tested products caused concerning enamel erosion.

SUMMARY

Aim: The study evaluated the efficacy and potential erosion of non-peroxide strips compared to hydrogen peroxide (HP) whitening strips (WSs).

Methods: Color evaluation samples (N=64) were distributed into four groups and treated according to manufacturer's directions. NC: Negative control treated with water; BT: Non-peroxide Brilliant Dissolving Strips; FM: Non-peroxide Fancymay Teeth WSs; WS: Crest 3D Brilliance HP White Strips. A contact-type spectrophotometer was used to measure color at baseline (T1), 1-day posttreatment (T2), and 1-week posttreatment (T3). Teeth were cut to a rectangular block for micro-CT erosion assessment. The samples (N=30) were divided into five groups. In addition to the four groups for color assessment, a positive control (PC) treated with 0.25% citric acid was added. The samples were scanned, reconstructed, and measured for erosion depth using a micro-CT analysis program software. Kruskal-Wallis test was used to determine differences in color change and erosion depth among the groups. Tests of

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hypotheses were two-sided with an alpha level of 0.05.

Results: The mean ΔE^*_{ab} at 1-day/1-week posttreatment were 2.4/2.5, 2.8/2.9, 2.8/3.2, and 8.6/11.0 for NC, BT, FM, and WS, respectively. There was a statistically significant difference for ΔE^*_{ab} at 1-day and 1-week posttreatment ($p<0.001$). Group WS had the highest color change, while the other three groups did not differ from each other ($p>0.05$). Mean erosion depths in microns were 0.52, 0.58, 0.42, 0.49, and 29.55 for NC, BT, FM, WS, and PC, respectively. There was a statistically significant difference among the groups ($p=0.004$). Group PC had the greatest erosion, while the other groups had negligible erosion that did not differ from each other ($p>0.05$).

Conclusion: Peroxide WSs had superior whitening efficacy compared to non-peroxide strips. None of the tested products compromised tooth structure integrity through enamel erosion.

INTRODUCTION

The desire to have a sparkling and white smile has driven the growth of the global whitening market. In 2019, the tooth whitening market was valued at $1.7 billion in the United States, and is further expected to grow substantially to exceed $2 billion in 2024.[1] Historically, tooth bleaching started as an in-office procedure with the use of a highly concentrated hydrogen peroxide (HP) solution requiring meticulous protection of the oral cavity by the dentist.[2] The use of a customized tray and carbamide peroxide enabled the bleaching procedure to be performed at home under the supervision of an oral health care professional.[3] Trays continue to be one of the most favorable delivery systems but were recognized to be time consuming in fabricating and also demanded significant compliance of the users. A major innovation that increased the use of over-the-counter (OTC) whitening products was the delivery of HP by strips that could be easily applied to the teeth. Thus, whitening strips (WSs) became the most popular, because of their convenience, low cost, less possible damage to the gingiva, and good esthetic results.[4]

Regardless of the bleaching technique and delivery system, the most recognized agent that causes bleaching is HP. HP when applied to the outer tooth surface readily penetrates into the tooth, interacts with stain molecules within the enamel and dentin, and may also alter the surface microscopically resulting in an increase in lightness and decrease in chroma.[5] Despite the successful use for more than 100 years, there have been concerns on the use of highly concentrated HP and its potential to induce intense inflammation in the pulp tissue.[6-8]

Additionally, the EU Council Directive 2011/84/EU stated that "Tooth whitening or bleaching products containing concentrations greater than 0.1% or less than 6% of HP are to be only sold to dental practitioners, which further promoted the search for non-peroxide whitening products and opened the market for other ingredients such as sodium hypochlorite, activated charcoal, citric acid, and phthalimido peroxy caproic acid (PAP).[9,10] Among these, sodium chlorite reacts with the citric acid and generates chlorine dioxide as an active bleaching agent.[11] Sodium hypochlorite, similar to HP in mechanism, oxidizes the double bonds within the chromogen structure.[12]

With the increase of non-peroxide whitening products on the market, there are uncertainties of their effects on enamel erosion and if the desired whitening results are indeed comparable to HP. The International Organization for Standardization (ISO) creates documents that provide requirements and guidelines to ensure that materials and products are fit for their purpose.[13] The ISO 28399 standard outlines test methods for laboratory assessment of tooth bleaching efficacy and safety requirements.[14] Based on the standard, there are no specific thresholds for bleaching efficacy, but the erosion created by a bleaching product should be less than erosion caused by 0.25% citric acid used for 4 hours. Thus, the purpose of this study was to evaluate two types of non-peroxide WSs compared to an ADA Seal of Acceptance—HP strips product in terms of whitening efficacy and enamel erosion.[15] We hypothesized that there would be no difference in efficacy and enamel erosion depth among the different experimental groups.

METHODS AND MATERIALS

Sample Selection and Preparation

Extracted sound human third molars (N=94) were collected and stored in 0.2% sodium azide solution at 4°C. Teeth were cleaned of gross debris and placed in artificial saliva at room temperature. Artificial saliva was prepared according to Shellies and others, and replaced weekly throughout the study.[16] Extracted molars were distributed into two major parts. A total of 64 teeth were used for tooth color evaluation (16 specimens/group), while another 30 teeth were used for erosion evaluation (6 specimens/group).

Experimental Groups for Color Evaluation

Prepared specimens were allocated into four groups based on severity of tooth discoloration, and bleaching materials were used according to manufacturers' directions. NC: Negative control treated with Grade 3 water for 60 minutes; BT: Non-peroxide Brilliant Dissolving Strips (Lornamead Ltd, Wiltshire, UK), applied 5 minutes twice a day for 7 days; FM: Non-peroxide Fancymay Teeth WSs (Shenzhen Hanyun Technology Co Ltd, Shenzhen, Guangdong, China), applied 60 minutes each day for 14 days; WS: Crest 3D Brilliance HP White Strips (Procter & Gamble, Cincinnati, OH, US), applied 30 minutes each day for 16 days. To apply, WSs were removed from their liner and placed with the gel side to the buccal surface, slightly pressed against the teeth for the best contact, and the remainder folded onto the occlusal surface. Table 1 summarizes the information for the bleaching materials.

Tooth Color Change Assessment

The step-by-step procedure from specimen preparation to instrumental color assessment is outlined in Figure 1. The roots were trimmed 3-mm apical to the cemento-enamel junction and the pulp was removed. Teeth were mounted on the top of a plastic dish with cyanoacrylate adhesive (Super Glue Liquid, 3M, St. Paul, MN) and further stabilized with acrylic resin on the lingual side. One operator performed the instrumental color measurements on the middle-third of the buccal surface using a contact type intraoral spectrophotometer (VITA Easyshade Compact, VITA GmBH, Bad Sackingen, Germany). A custom fabricated jig was used for repeated measurements on the same area. Measurements were performed under a color-controlled light box (MM 4e GTI Mini Matcher, GTI Graphic Technology, Inc, Newburgh, NY, USA) at CIE D65, a color temperature of 6500 K and light intensity of ≈1200 lux. Results were gathered by recording L^*, a^*, and b^* values at baseline (T1), 1-day posttreatment (T2), and 1-week posttreatment (T3). The overall color change, as measured with the spectrophotometer, was expressed as ΔE_{ab}^* from the Commission Internationale de l'Eclairage (CIE 1986).[17] The following equation was used and calculated relative to baseline color parameters (L^*1, a^*1, b^*1): $\Delta E_{ab}^* = [(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2]^{1/2}$

Table 1: *Summary of Whitening Materials Used*

Group	Brand Name	Listed Ingredients
NC	N/A	Water of grade 3
BT	Brilliant Dissolving Strips	Accelerator ingredients: Aqua, Sodium Chloride, Whitening Strip (WS) ingredients: PVP, Glycerin, Aqua, Citric Acid, Aroma, Polysorbate-80, Sucralose, Propylene Glycol, Cellacefate, Maltodextrin
FM	Fancymay Teeth WSs	Glycerin, Aqua, Cellulose Gum, Sodium Chlorite, Disodium EDTA, Cocos Nucifera oil, Citric Acid, and D. L-menthol
WS	Crest 3D Whitestrips Brilliance White	PVP, PEG-8, Water, HP, Acrylates Copolymer, Sodium Hydroxide, and Sodium Saccharin

Abbreviations: NC, Negative control treated with water; BT, Non-peroxide Brilliant Dissolving Strips; FM, Non-peroxide Fancymay Teeth Whitening Strips; WS, Crest 3D Brilliance Hydrogen Peroxide White Strips; HP, hydrogen peroxide.

On completion of baseline color measurements, the teeth were treated with WSs according to manufacturers' directions. The teeth were stored in artificial saliva at room temperature throughout the experimental time period.

Micro-computed Tomography (Micro-CT) for Erosion Assessment

The step-by-step procedure from specimen preparation to micro-computed tomography (micro-CT) assessment

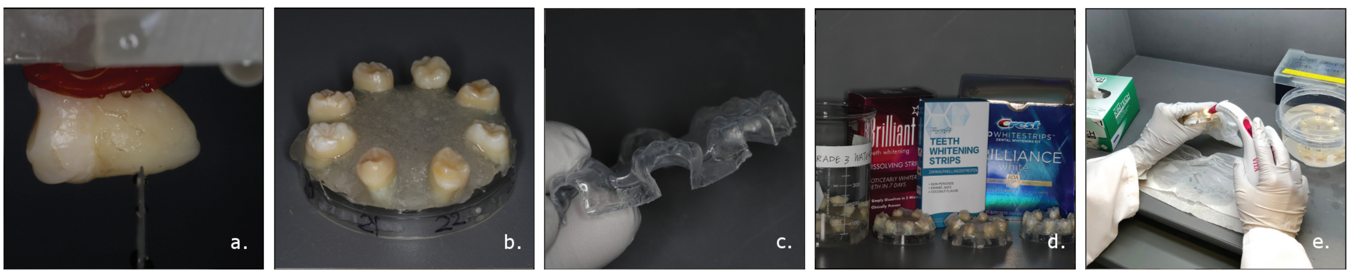

Figure 1. *Step-by-step protocol for bleaching efficacy assessment.(a) Root trimming; (b) Teeth mounting; (c) Jig fabrication; (d) Whitening treatment; (e) Instrumental color measurement.*

is outlined in Figure 2. Enamel blocks were prepared from caries-free human extracted molar teeth (N=30). Teeth were cut to a rectangular shape of 4×4×6 mm and mounted on acrylic rods with cyanoacrylate adhesive (Super Glue Liquid). The buccal enamel surfaces were polished with medium-grit paper and then sequentially polished up to P1200 paper. The flat surfaces were painted with nail-varnish (Sally Hansen, New York, NY, USA) to expose a flat 2×4-mm window. Care was taken to prevent dehydration of test specimens during the specimen preparation procedure. The specimens were randomly distributed into five groups of six specimens each. In addition to the four groups (NC, BT, FM, WS) for tooth color measurements, a positive control (PC) group was added that consisted of treatment with 0.25% citric acid (pH=3.68) for 4 hours, as per ISO 28399 guidelines.[14] Tooth whitening was performed according to manufacturers' direction on the exposed window the same way as for the color evaluation samples.

On completion of treatment, all specimens were scanned using the SkyScan 1272 desktop micro-CT system (Bruker micro-CT NV, Kontich, Belgium), with an accelerating source voltage of 100 keV, a source current of 100 mA, and an exposure time of 2600 ms. All the specimens were positioned in the same way in the center of rotation of the mounting device. During the scanning process, the samples were rotated at 180°, with an imaging voxel size of 4.5 μm and rotation step of 0.4. The images were saved as 16-bit Tagged Image File Format (TIFF) files and consequently exported to a reconstruction program (NRecon software, version 1.7.4.6; SkyScan) for the reconstruction of the 3D object. The tomographic reconstruction produced a dataset of slice views in 16 bit TIFF format, which were assessed in the analysis program (CTAn software, version 1.18.8.0; SkyScan). Figure 3 illustrates the tomographic reconstruction and digital slicing of the sample perpendicular to the occlusal surface at the middle slice. Lesion depths were assessed in the middle slice of each sample, and three vertical measurements from lesion surface to upper and bottom surface were recorded and averaged. One operator that was blinded to the treatment groups performed the reconstruction and measurements.

Statistical Analysis

G*Power 3.1.9.4 (Heinrich-Heine Dusseldorf University, Germany) was used to determine the sample size based on the following parameters: 80% power, 2.7 effect size, SD of 1.1, and four experimental groups. A minimum sample size of 16 specimens per group was assessed to be appropriate. Measurements for tooth color assessment included L^*, a^*, b^*, ΔL^*, Δb^*, and ΔE_{ab}^*. A sample size of six specimens per group were used for the erosion assessment per ISO 28399 guidelines. Kruskal-Wallis procedure was used to determine significant differences in color change and erosion depth among the groups. Dwass-Steel-Critchlow-Fligner pairwise comparisons were used when needed. Tests of hypotheses were two-sided with an alpha level of 0.05. Analysis was conducted with SAS v 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

The baseline lightness (L_1) of teeth ranged from 74.1 to 85.3, with a mean value of 79.9. Baseline chroma in the yellow-blue ranged from 16.0 to 34.2, with a mean value of 25.78. There was no statistically significant difference in any color parameter among the four groups (L_1, a_1, and b_1) at baseline ($p>0.05$, in all instances).

The overall color change (ΔE^*_{ab}) relative to baseline over time by group are summarized in Table 2 and illustrated in Figure 4 as boxplots. The magnitude of ΔE^*_{ab} was based on an increase in lightness and decrease in chroma of a^* and b^*. The mean ΔE^*_{ab} at 1-day/1-week posttreatment were 2.4/2.5, 2.8/2.9, 2.8/3.2, and 8.6/11.0 for NC, BT, FM, and WS, respectively. There was a statistically significant difference among the four groups for ΔE^*_{ab} at 1-day and 1-week posttreatment ($p<0.001$, in both instances). Group WS had the highest color change regardless of timepoint, while the other three groups did not differ from each other ($p>0.05$, for all pairwise comparisons). The "ISO/TR 28642"

Figure 2. *Step-by-step protocol for micro-CT erosion assessment. (a) Sample mounting; (b) Sample polishing; (c) Varnish painting; (d) Whitening treatment; (e) Micro-CT scanning.*

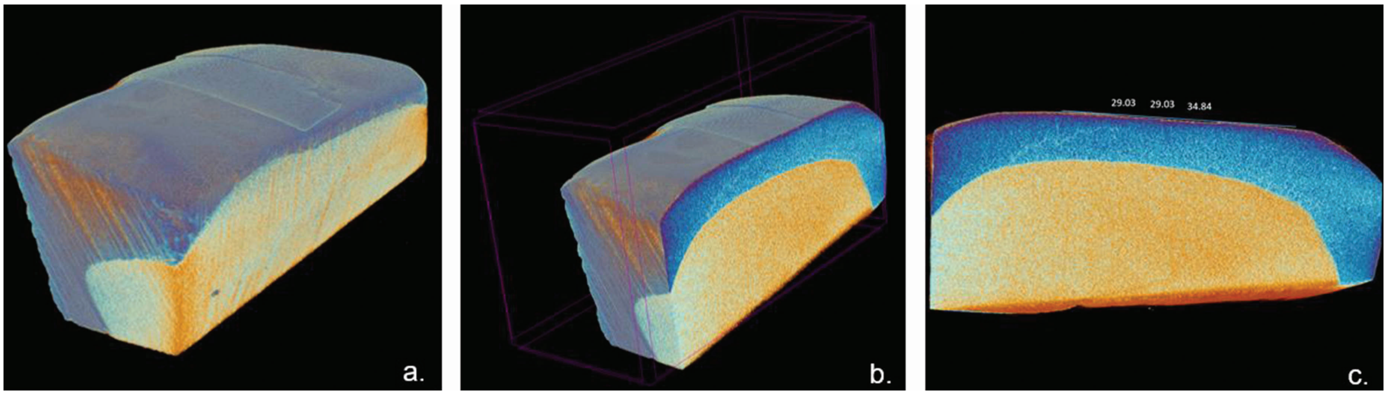

Figure 3. *Step-by-step procedure for erosion measurement. (a) 3D reconstruction of sample; (b) Digital slicing at middle slice; (c) Three vertical measurements from lesion surface to upper and bottom surface.*

Table 2. *Overall Color Change by Group Over Time (Mean±SD)*[a]

Group	NC	BT	FM	WS	*p*-value
ΔE*2-1	2.4 ± 1.3 a	2.8 ± 1.2 a	2.8 ± 1.4 a	8. 6± 1.9 b	<0.001
ΔE*3-1	2.5 ± 1.5 a	2.9 ± 1.8 a	3.2 ± 2.3 a	11.0 ± 2.9 b	<0.001

Abbreviations: NC, Negative control treated with water; BT, Non-peroxide Brilliant Dissolving Strips; FM, Non-peroxide Fancymay Teeth Whitening Strips; WS, Crest 3D Brilliance Hydrogen Peroxide White Strips.
[a] *Within rows, different lowercase letters indicate means that are statistically different after pairwise comparisons (*p*<0.05).*

outlines the definition of thresholds that can also be used as a reference to determine bleaching efficacy.[18] Based on the report, perceptibility threshold (PT) ΔE^*ab=1.2, is the difference in color that can be detected by 50% of observers, with the other 50% of observers noticing no difference in color between the compared objects while acceptability threshold (AT) is a difference above ΔE^*ab=2.7, where 50% of observers would consider the compared objects to be an unacceptable match. All groups exceeded the perceptibility threshold of ΔE^*_{ab}=1.2 at both timepoints.[18] Group NC was the only group that did not exceed the acceptability threshold of ΔE^*_{ab}=2.7 at both time points.[18]

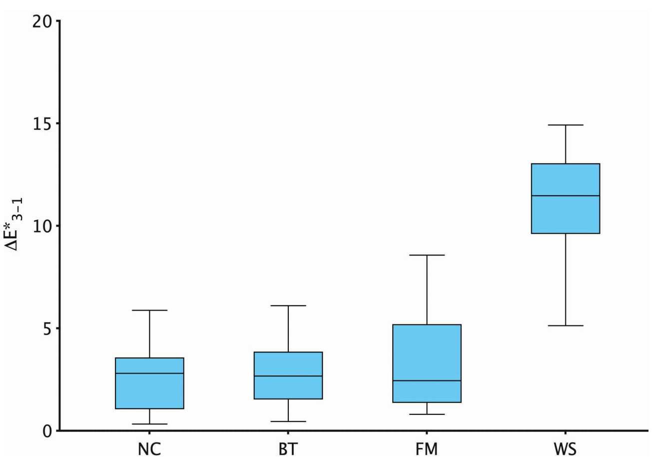

Figure 4. *Boxplots of overall color change by group at 1-week posttreatment.*

The mean erosion depths by group are summarized in Table 3 and illustrated as boxplots in Figure 5. The mean erosion depths in microns were 0.52, 0.58, 0.42, 0.49, and 29.55 for NC, BT, FM, WS, and PC, respectively. There was a statistically significant difference among the five groups (p=0.004). Group PC had the greatest erosion depth, while the other groups had negligible erosion that did not differ from each other (p>0.05, for all pairwise comparisons).

DISCUSSION

Non-peroxide dental whitening is marketed increasingly as a result of the scientific community's effort to develop innovative whitening materials. The increase is also driven by regulatory guidelines limiting the allowable concentration of HP. Manufacturers claim that such non-peroxide-based products result in instant whitening while having minimal adverse effects. However, there is scarce literature to support these claims, which leaves oral health care professionals undermined in advising the public on effective and safe whitening products.

Table 3: *Erosion Depth (µm) By Group (Mean±SD)*[a]						
GROUP	**NC**	**BT**	**FM**	**WS**	**PC**	***p*-value**
Depth	0.52 ± 0.19 a	0.58 ± 0.20 a	0.42 ± 0.16 a	0.49 ± 0.17 a	29.55 ± 3.52 b	<0.001

Abbreviations: NC, Negative control treated with water; BT, Non-peroxide Brilliant Dissolving Strips; FM, Non-peroxide Fancymay Teeth Whitening Strips; WS, Crest 3D Brilliance Hydrogen Peroxide White Strips.

[a] *Within rows, different lowercase letters indicate means that are statistically different after pairwise comparisons (*p*<0.05).*

This *in vitro* study was designed to evaluate the whitening efficacy and potential erosion of two types of non-peroxide WSs relative to a negative control and an OTC WS holding an ADA Seal of Acceptance.

Based on the results, we rejected the first null hypothesis. There was a significant difference in overall color change at 1-day and 1-week posttreatment, with the ADA Seal of Acceptance OTC whitening strip (WS) exhibiting the highest color change. The color change associated with non-peroxide WSs containing sodium chloride, sodium chlorite, and citric acid were not different from the negative control.

The negative control of water was included to add rigor to the study design, and it is important to note that the mean ΔE^*_{ab} of the negative control was close to the acceptability threshold (ΔE^*_{ab}=2.7). This is in alignment with a systematic review of *in vitro* studies that calculated an estimate of ΔE^*_{ab} = 2.9 for negative controls.[19] Thus, the tested non-peroxide WSs had comparable color change as to the use of water. The study results support the findings by Kielbassa and others that reported negligible color change with non-peroxide whitening kits.[11] However, our results are in discordance with a study that used the same product "Brilliant 5-minute kit" and reported a significant color change with the non-peroxide OTC product.[10] There may be several reasons for the discrepancy. The Cohen and others study pretreated extracted teeth with a staining solution, measured tooth color visually with a shade guide, and used a small number of teeth for each group.

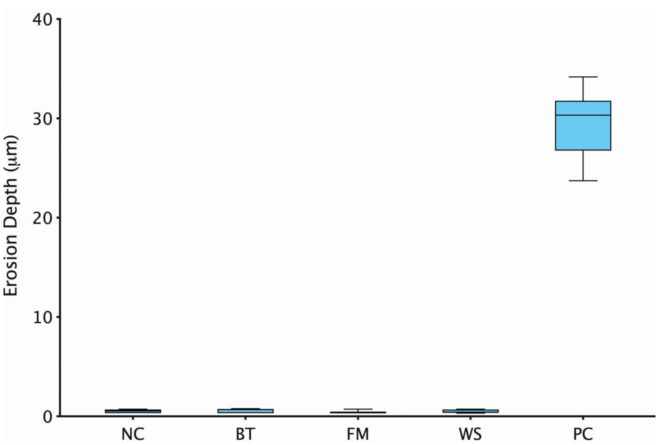

Figure 5. *Boxplots of erosion depths by group.*

The mean ΔE^*_{ab} = 11.0 of the Crest Brilliance WS in our study was comparable to another study that evaluated a similar Crest WS and reported a mean ΔE^*_{ab} = 10.0 at 1-week posttreatment.[20] The efficacy results of the current study prompted the need to evaluate the mechanism of non-peroxide whitening agents. The mechanism of HP has been extensively studied and is based on well-documented diffusion of HP into the tooth structure that reaches the pulp within 5-15 minutes.[21] During the course of diffusion, the active oxygen radicals interact with the chromophores, resulting in lightening of the tooth.[5] Thus, further studies on the mode of action of non-peroxide whitening agents to support their efficacy are warranted.

There is no dispute that the two most commonly reported side effects of tooth whitening are tooth sensitivity and gingival irritation.[22] With the increased use of OTC products that are not supervised by oral heath professionals, there have also been concerns about tooth structure integrity such as changes to surface roughness, microhardness, and loss of dental hard tissues associated with an acid attack not from bacterial origin, which is defined as erosion.[14,23-27]

Based on the results, we rejected our second null hypothesis. There was a difference among the tested groups with the positive control of 0.25% citric acid for 4 hours, showing an erosion depth of approximately 30 microns. This is comparable to another study that used 1.0% citric acid for 1 hour and reported enamel loss of 24 microns.[28] All tested WSs, regardless of composition, had negligible erosion, which was comparable to the use of water. This is in agreement with a study that found that whitening did not increase the susceptibility of enamel to erosion.[25] To our best knowledge, this is the first study that compared erosion depth of non-peroxide versus peroxide WSs using micro-CT. Micro-CT was used to enable 3D reconstruction of the samples and digitally slicing the samples for measurements. The results are significant in informing users that OTC products, when used according to manufacturer's directions they do not cause potential erosion to the enamel. The limitations of this study include the lack to fully replicate the dynamics of the *in vivo* oral environment and not evaluating whether erosion may have been detected with potential overuse of the products.

CONCLUSIONS

The study evaluated the efficacy and safety of non-peroxide strips compared to HP WSs. Within the limitations of the study, we conclude that peroxide WSs had superior whitening efficacy compared to non-peroxide strips. None of the tested products compromised tooth structure integrity by potential enamel erosion. Future studies should further evaluate other aspects of safety such as changes in microhardness, surface roughness, and diffusion into the tooth. Additionally, the effect of adaptation of various strip systems relative to color change and adverse effects could be explored.

Acknowledgement

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Regulatory Statement

The use of extracted teeth without identifiers was determined to be nonhuman subject research by the local human subjects oversight committee.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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