International Journal of Research in Health and Allied Sciences

Journal home page: www.ijrhas.com

Official Publication of "*Society for Scientific Research and Studies*" [Regd.]

ISSN 2455-7803 Index Copernicus value 2016 = 68.10

Original Article

Effect on Pulp Chamber by Penetration of Carbamide Peroxide: An *in vitro* Study

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ABSTRACT

Purpose: Vital tooth bleaching has become a popular procedure for whitening teeth. Most home bleaching products contain 10% carbamide peroxide. The purpose of this in vitro study was to measure the quantity of hydrogen peroxide that reaches the pulp chamber from three carbamide peroxide products: Opalescence™, Sparkle™, and Rembrandt™. **Materials and Methods:** Seventy roots of extracted premolars were amputated approximately 3 mm apical to the cementoenamel junction, and the pulp tissues were removed. They weredivided into three experimental groups (n = 20) and a control group of 10 teeth. An acetate buffer solution was placed in the pulp chamber before the crown was exposed to the bleaching agent at 37°C for 25 minutes. The buffer solution was removed and reacted with leuko crystal violet and horsera dish peroxidase. The optical density of blue colour that developed was measured at a wave length of 596 nm and read from a standard curve for hydrogen peroxide quantity. **Results:** The measured amounts of hydrogen peroxide were 3.605 = 1.405, 1.282 = 0.762, and0.339 f 0.251 µg for the Opalescence™, Sparkle™, and Rembrandt™ groups, respectively. A statistically significant difference in the hydrogen peroxide levels was observed by analysis of variance (p <.05) among the three groups. **Conclusion:** It was concluded that the penetration of commercial bleaching products was different even though the products were labelled as having the same 10% carbamide peroxide.

Key words: Carbamide Peroxide, tooth bleaching, vital tooth.

Received: 12 May 2018 Revised: 18 June 2018 Accepted: 20 June 2018

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This article may be cited as: Talesara K, Khatri M. Effect on Pulp Chamber by Penetration of Carbamide Peroxide: An in vitro Study. Int J Res Health Allied Sci 2018; 4(4):46-49.

INTRODUCTION

Vital tooth bleaching has become a conservative means to whiten discoloured teeth. The in-office techniques using 30 to 35% hydrogen peroxide have been advocated for many years. More recent techniques allow patient-application of bleaching agents outside the dental office, so called "at-home bleaching". The materials most commonly used for at-home bleaching techniques include a 10 to 15% carbamide peroxide or 2 to 10% hydrogen peroxide concentrations in a viscous gel. A 10% carbamideperoxide solution contains 3.62%hydrogen peroxide and 6.38%urea.' Theoxygenating property ofhydrogen peroxide can lighten teethif the material is placed inside acustom tray and held in contact with the teeth for a period of time. With early generation bleaching materials, results generally were seen in 2 to 3 weeks and the final outcome was complete in *5* to 6 weeks.[2] Current materials work even more quickly. Manufacturers recently have introduced bleaching products that are dentist-prescribed for patients to apply at home, as well as "over-the-counter" bleaching kits that are sold directly to customers.

Numerous studies evaluating the safety and effectiveness of current bleaching techniques and applicationshave been reviewed by Haywood.[3] The use of carbamide peroxide has been reported to cause tooth sensitivity to temperature change.[4] Studies that evaluated differentconcentrations of carbamideperoxide and hydrogen peroxidehave shown that hydrogen peroxide,whether applied directly or derivedfrom carbamide peroxide, passesthrough the enamel and dentin tothe pulp chamber of freshlyextracted teeth.[5,6]. The majority ofthe products currently used forat-

homebleaching contains a 10%carbamide peroxide solution[3]. Fewdata are available regarding thedifferences in diffusion rates of thecommercial bleaching gels. Thisstudy was designed to observe thepenetration of hydrogen peroxideinto the pulp chamber from threebrands of tooth-bleaching gels containingequal concentrations of carbamide peroxide, using extracted human teeth.

Each product represents a different clinical approach. Opalescence™(Ultradent Products, Salt Lake City, Utah) is a high viscosity gel that is recommended for use primarily at night during sleep. Rembrandt™ (Den-Mat, Santa Maria, California) is a medium viscosity gel. It is recommended for use 1 to 4 hours per day with new gel added to the tray every hour. Both of these products are dentist-supervised and dispensed. Both procedures also use a custom-made vacuum-formed tray as the vehicle to carry the bleaching gel to the mouth. Sparkle™ (Kuron Health Products Corp., Oakland, California) is an over-the-counter bleaching kit. The medium viscosity gel has a 1/2 to 1-hour application period, and the kit includes a "boil and bite tray." All three bleaching gels used in this study contain 10% carbamide peroxide.

MATERIALS AND METHODS

Intact, non-restored, and non-carious human maxillary premolars freshly extracted for orthodontic purposes were collected. After extraction, the teeth were placed in *0.05%* sodium hypochlorite for a few days to retard bacterial growth and thereafter were stored in saline until used. Seventy teeth of equal size were selected and divided into threeexperimental groups with 20 teeth per group and a control group of 10 teeth. The teeth were cleaned with pumice and then sectionedapproximately 3 mm incisal to the cementoenamel junction, and the pulpal tissue was removed. The pulp chambers were prepared to thesame diameter with a No. 016 roundbur. The external root surface ofeach tooth, including a part of thecrown, was covered with two layersof coloured nail polish. The crown ata level of 3 mm gingival to the marginalridges was left uncovered.

Each experimental group comprised a sample size of 20 teeth. Teeth were exposed to commercially available carbamide peroxide bleaching gels: (1) Opalescence, (2) Sparkle, or (3) Rembrandt, all of which contain 10% carbamide peroxide. Acetate buffer (25 µL of 2M) was placed into each pulp chamber to act as a stabilizing medium for any hydrogen peroxide that diffused into the pulp chamber. Then the crown was placed in contact with the peroxide gel at the level of coloured nail polish for 25 minutes at 37°C (Figure 1). Control specimens were submerged in distilled water instead of peroxide gel under the same conditions. At the end of the exposure period, the acetate buffer was removed from the pulp chamber by micropipette and placed in a 5-mL volumetric tube. The pulp chamber was rinsed twice with a 100 µL portion of distilled water, and the rinsing was added to the test solution.

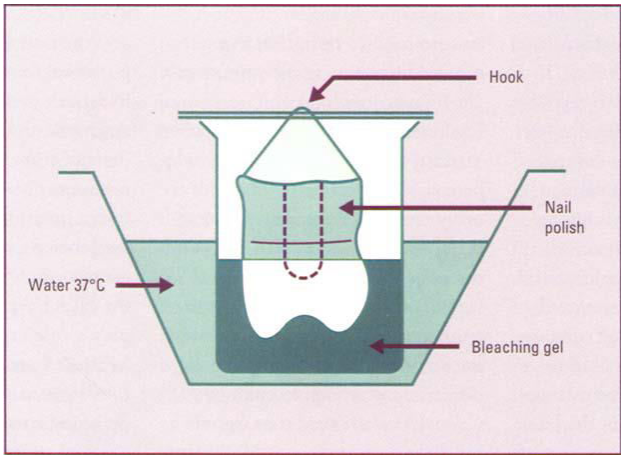

Figure 1: Diagram of placement of tooth in contact with bleaching gel.

The quantities of hydrogen peroxide that diffused through the crown were analysed colourimetrically, using the leuko crystal violet/horseradish peroxidase assay described by Mottola et al.[7] Leucocrystal violet (100 µL,0.5 mg/mL) and horseradish peroxidase(50 µL, 1 mg/mL) were addedto the test solution and diluted to3.0 mL. The optical density of the blue color that developed was measured in a Spectronic601 (MiltonRoy Co., Rochester, New York) ultraviolet spectrophotometer at awavelength of 596 nm. The amountof hydrogen peroxide in the test sampleswas determined by comparingthem with a standard curve generatedby known amounts of hydrogen peroxide.Fivereplicate experimentsper each dilution of hydrogen peroxidewere performed. The calibration curves were made with known amounts of hydrogen peroxide, ranging from 0.5 to 5 µg. A straight line can be drawn through these data.

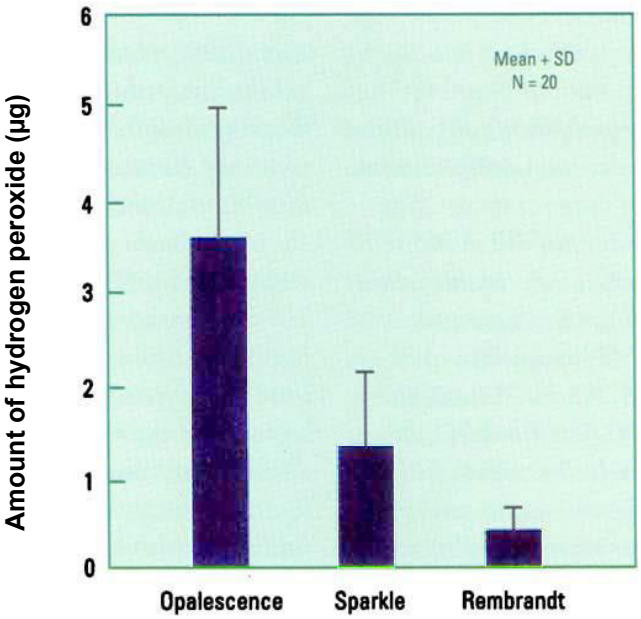

Figure 2: Bar graph illustrating amount of Hz02 diffused into the pulp chamber of teeth in contact with different bleaching gels.

RESULTS

Carbamide peroxide readily diffused through the coronal wall to the pulp chamber of the test samples. The results are summarized in Figure 2. Opalescence™ produced the greatest quantity of hydrogen peroxide (3.605 ±1.405 µg), followed by Sparkle™ (1.282 ± 0.762 µg), and Rembrandt™ (0.339 ± 0.251 µg), respectively. In the control teeth, the hydrogen peroxide was negligible. Analysis of variance, followed by Scheffe's test for the computation of confidence intervals, showed statistically significant differences among all groups ($p<.05$).

DISCUSSION

Numerous methods are available for the spectrophotometric determination of hydrogen peroxide in microgram amounts. The method used in this study involves colourimetric analyses using leukocrystal violet and horseradish peroxidase assay as described by Mottola et al.[7] It is a simple test, and the apparatus needed is generally available.

The experiment conditions chosen for this study were similar to the conditions reported by Bowles and Ugwuneri and Cooper et al.[5,6] To attain the precision of the method, five replicate experiments were performed for each dilution of hydrogen peroxide to produce a calibration curve. The absorbance readings for the control sample (against purified water as reference) with the sample exposed to laboratoryfluorescent light and daylight showedno change for at least 3 hours. Toattain greater accuracy, **two** replicateexperiments were performed usingthe control and the testsolutions.

The dentin thickness and surfacearea that contacted the bleachinggels also were taken into consideration.Dentin thickness could not bemeasured directly. However, teethof similar size were chosen, and abur of the same size was used toprepare the pulp space; the assumptionwas made that dentin thicknessalso was similar. The smear layerwas not removed from the pulpalwalls before testing the hydrogenperoxide diffusion. Since all pulpchambers were prepared in a similar manner, it was decided that there was no need to open the dentinal tubules by removing the smear layer.

Hydrogen peroxide, whether applied directly or derived from carbamide peroxide indirectly, has been previously reported to readily penetrate the coronal walls of teeth and enter the pulp chamber.[5,6] Bowles and Ugwuneri revealed that elevation of temperature significantly increased the amount of hydrogen peroxide diffusion into the pulp chamber.[5] Cooper et al showed that significantly less peroxide reaches the pulp from a carbamide peroxide source than from free hydrogen peroxide.[6] The rate of diffusion of hydrogen peroxide through the coronal wall of the tooth is somewhat limited and the rate of diffusion is not proportional to the concentration of hydrogen peroxide. Results of this study show that hydrogen peroxide derived from carbamide peroxide products does reach the pulp in differentquantities even though they are labelled as having the same concentration. Other components of the bleaching materials also may play a role in penetration of the hydrogen peroxide through the tooth structure. Most carbamideperoxidecontaining products also contain carbopol (carboxy polyethylene polymer), glycerine, sodium stearate, and flavouring agents.[1]

Carbopol has been added to most commercially available carbamide peroxide gels to increase gel viscosity and delay oxygen release.[8] Opalescence™ contains a higher concentration of carbopol, to increase both thickness and activity time. Rembrandt™ contains a rnoderate concentration of carbopol, and it is not as thick as Opalescence™. Both products are in a glycerine base. Most over-the-counter materials contain 0.5 to 1.5% carbopol.[1] Sparkle™ is thicker than Rembrandt™and thinner than Opalescence™. Therefore, the results of this study appear to indicate that the more viscous the gel, owing to the added carbopol, the more penetration of hydrogen peroxide into the pulp chamber occurs. Rembrandt™(medium viscosity) exhibited lower hydrogen peroxide diffusion into the pulp chamber. This tends to confirm a similar report demonstrating the diffusion of hydrogen peroxide through a dentin disk into a pulp chamber in-vitro.[9] Products with higher carbopol concentration appear to perform better clinically, since the bleach stays active longer and further thickening reduces bleach loss from the tray.[1]

Hanks et al, who indicated that the diffusion of hydrogen peroxide is complex, also stated that the permeability coefficients for all hydrogen peroxide-containing bleaching agents are not of the same magnitude.[9] At least two forces might be working against the diffusive flux of molecules from the bleaching agents toward the pulp: (1) convection due to positive pulpal pressure and (2) osmotic pressure of the gels. However, the results from the presentstudy probably were not affected by pulpal pressure, because the pulp was absent and no intrapulpal pressure was simulated. In vital teeth, penetration of hydrogen peroxide would decrease when dentin was exposed and the smearlayer was removed, because there would be an outward flow of fluid opposing inward diffusion.[10-12]

Several studies examining the safety of bleaching agents can be difficult to interpret, because clinical tooth - hypersensitivity is a common side effect experienced by some people who undergo the tooth bleaching process.[13] The safety and efficacy of bleaching techniques should be based not only on the product itself but also on the delivery method and treatment time employed, especially for those that are used without the direct supervision of a dentist.

CONCLUSION

The penetration into the pulp chamber of three commercial bleaching gels containing 10% carbamide peroxide was tested in extracted human teeth. Opalescence™ produced the greatest quantities of hydrogen peroxide in the pulp, followed by Sparkle™ and Rembrandt™, respectively. It was concluded that the rate of diffusion of these gels is different, although they contained the same peroxide concentration. It may imply that equal concentration of 10% carbamide peroxide

products may have different sensitivity and efficacy rates in tooth bleaching, owing to other additives in the bleaching materials.

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Source of support: Nil **Conflict of interest:** None declared