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Original Article

Dentistry Section

An in Vitro Spectrophotometric Analysis of the Penetration of Bleaching Agent into the Pulp Chamber of Intact and Restored Teeth

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ABSTRACT

Aim: To investigate the pulp chamber penetration of bleaching agent in intact teeth and teeth following restorative procedure.

Methodology: Sixty extracted human incisors were selected and divided into 6 Groups. Four Groups were restored with hybrid composite resin and resin modified glass ionomer cement respectively, while 2 Groups were left intact. The Groups were then immersed in the treatment agents for 60 mins at 37°C. The optical density of the resultant blue solution obtained by adding leucocrystal violet and horseradish peroxidise to the acetate buffer solution present in the pulp chamber was measured spectrophotometrically and the data thus obtained was statistically analysed using ANOVA and paired t-test.

Results: The amount of pulpal peroxide penetration in restored teeth was significantly higher than intact teeth ($p < 0.001$). the control Group showed no peroxide penetration. Groups restored with resin modified glass ionomer cement showed higher pulpal peroxide levels than Groups restored with hybrid composites, though the difference was not statistically significant ($p = 0.52$).

Conclusion: Peroxide readily penetrates into the pulp through intact and restored teeth, with restored teeth showing higher pulpal peroxide levels than intact teeth. Teeth restored with resin modified glass ionomer cement showed higher pulpal peroxide level than teeth restored with composite resins.

Keywords: Carbamide peroxide, Bleaching, Pulp, Hybrid Composite Resin, Resin modified glass ionomer cement

INTRODUCTION

Vital tooth bleaching with gel technique has become popular in dental procedures. This technique involves both “at home” and “in office” bleaching techniques. Various concentrations of hydrogen and carbamide peroxide are used in this technique, of which 10% carbamide peroxide is the commonly used gel for “at home” bleach [1-3].

Hydrogen peroxide breaks down into free radicals that eventually combine to form molecular oxygen and water. The oxygen oxidizes the stained areas. The effects of carbamide peroxide are similar to hydrogen peroxide as it ultimately breaks down into urea and hydrogen peroxide [1,3,4,5].

In recent studies, it has been shown that both hydrogen peroxide and carbamide peroxide penetrate enamel and dentin and then the pulp and that the pulpal enzymes are significantly inhibited by hydrogen peroxide [4,6,7].

Composite resins are routine anterior esthetic restorative material but recently resin modified glass ionomer cement is also being used. When a bleaching process is applied to a restored tooth, the properties of the restorative material may be affected by the bleaching agent used [2,8-12].

The purpose of this in vitro study was to evaluate the amount of pulpal peroxide penetration of 10% carbamide peroxide through intact teeth and teeth restored with composite resin and resin modified glass ionomer cement.

MATERIAL AND METHODS

Sixty extracted human non-carious anterior maxillary teeth were used. The teeth were separated into 6 Groups of 10 teeth each.

In 40 teeth standardised class V cavities were prepared 2mm deep, 3mm in diameter and 2mm above the CEJ. The cavity margins were bevelled with a fine grit flame shaped diamond bur. The other 20 teeth were left intact (Group 1 and 2). Two Groups (Group 3 and 4) were restored with hybrid composite resin (Filtek Z350) and the other 2 Groups (Group 5 and 6) were restored with resin modified glass ionomer cement (Fuji II LC) in accordance with manufacturer’s instructions. After polymerization, setting the restorations were finished with soflex discs (3M) and stored for 24 hours in distilled water. The teeth were then subjected to thermocycling between 5° and 55°C for 100 cycles [2,4,5] [Table/Fig-1].

The roots of all teeth were sectioned 3mm apical to the CEJ and the pulpal tissue removed with a round bur, the pulp chamber was then rinsed with distilled water. An orthodontic wire was attached to each tooth with light cure composite resin. 100µl of 2M acetate buffer was placed into the pulp chamber of each tooth to Stabilise the hydrogen peroxide that might penetrate into the pulp [2,4,5].

Teeth were isolated using 2 layers of nail varnish, leaving a standardised buccal area exposed to the bleaching agents. For restored teeth, this area corresponded to 2 mm beyond the limits of the restoration. The intact teeth were treated in the same way [2,4,5].

Groups 1, 3 and 5 were immersed in distilled water and evaluated as control Group. Groups 2, 4 and 6 were immersed in 10% carbamide peroxide for 60 mins at 37°C [2,4,5].

The acetate buffer solution in the pulp chamber of each tooth was removed after exposure with Pasteur pipettes and transferred to a glass test tube. The pulp chamber of each tooth was then rinsed twice with 100µl portion of distilled water, placed that in the same glass test tube and was diluted with 3ml of distilled water. 100µl of 0.5mg/ml leukocrystal violet (sigma chemicals co.) and 50µl of 1mg/ml enzyme horseradish peroxidise (sigma chemicals co.) was also added to each test tube according to the method described by Mottola et al., [13]. This procedure was then repeated for each tooth [2,4,5].

The optical density of the resultant blue colour in the tubes was measured by a UV visible spectrophotometer at 596 nm wavelength and was converted to microgram equivalent of hydrogen peroxide [2,4,5]. The results of the measurements were statistically analysed using ANOVA and paired t-test.

Group 1	Consisted of 10 intact teeth immersed in distilled water
Group 2	Consisted of 10 intact teeth immersed in 10% carbamide peroxide.
Group 3	Consisted of 10 teeth restored with hybrid composite resin (Filtek Z350) immersed in distilled water.
Group 4	Consisted of 10 teeth restored with hybrid composite resin (Filtek Z350) immersed in 10% carbamide peroxide.
Group 5	Consisted of 10 teeth restored with resin modified glass ionomer cement (Fuji II LC) immersed in distilled water.
Group 6	Consisted of 10 teeth restored with resin modified glass ionomer cement (Fuji II LC) immersed in 10% carbamide peroxide.

[Table/Fig-1]: Test groups

RESULTS

The results are summarized in [Table/Fig-2] and the comparision between the Groups is shown in [Table/Fig-3] and [Table/Fig-4]. Groups 1, 3 and 5 in which teeth were immersed in distilled water acted as control. A statistically significant difference was seen in the pulpal peroxide penetration level between intact and restored teeth ($p < 0.001$), with intact teeth (Group 2) showing the least amount of pulpal peroxide penetration and the teeth restored with resin modified glass ionomer cement (Group 6) showing the highest pulpal peroxide level.

On comparison of peroxide penetration level between teeth restored with composite resin (Group 4) and teeth restored with resin modified glass ionomer cement (Group 6), although the results were not statistically significant ($p = 0.52$), the teeth restored with resin modified glass ionomer (Group 6) showed higher pulpal peroxide levels.

Group 1, in which intact teeth were immersed in distilled water, showed no pulpal peroxide level. Group 2, in which intact teeth were immersed in 10% carbamide peroxide, showed pulpal peroxide level in the range between 3.21 µg to 3.43 µg with a mean of 3.33 µg and a standard deviation of ± 0.08.

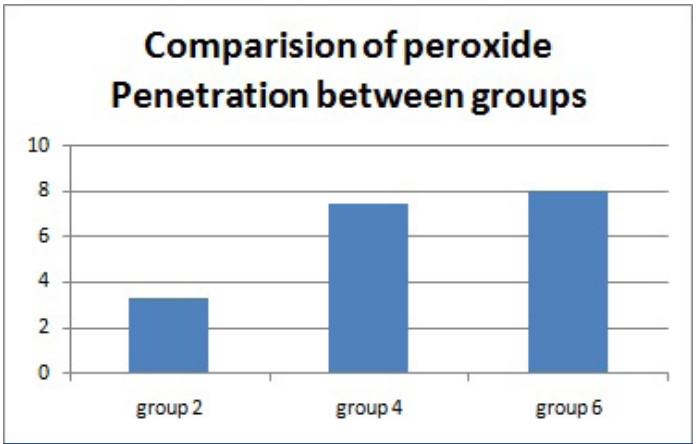
Group 3, in which teeth restored with a hybrid composite resin were immersed in distilled water, showed no pulpal peroxide level. Group 4, in which teeth restored with a hybrid composite resin were immersed in 10% carbamide peroxide, showed pulpal peroxide level in the range between 6.89 µg to 8.35 µg with a mean of 7.48 µg and a standard deviation of ± 0.49.

Group 5, in which teeth restored with a resin modified glass ionomer cement were immersed in distilled water showed no pulpal peroxide level. Group 6, in which teeth restored with a resin modified glass ionomer cement were immersed in 10% carbamide peroxide, showed pulpal peroxide level in the range between 6.39 µg to 8.50 µg with a mean of 8.00 µg and a standard deviation of ± 0.61.

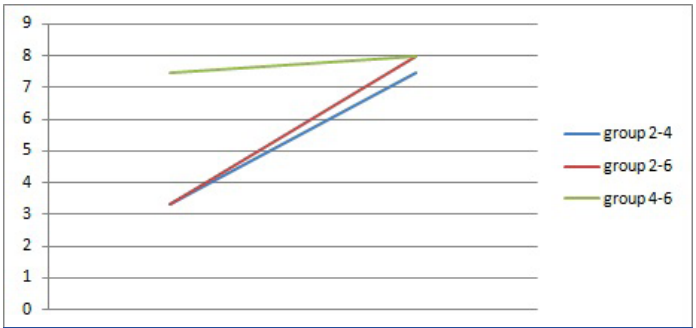
One way ANOVA was done for all 3 Groups. F-value was found to be 340.88 and $p<0.001$. This suggests the ANOVA test was highly significant.

Groups	Penetration (µg)		Difference Between Groups		
	Mean	SD	Groups Compared	t-value	p-value*
2. Intact teeth	3.33	0.08	2 – 4	28.72	p<0.0001, HS
4. Composite Restoration	7.48	0.49	2 – 6	24.01	p<0.0001, HS
6. RMGIC Restoration	8.00	0.61	4 – 6	0.646	p=0.52, NS

[Table/Fig-2]: Test data results and statistical analysis between groups
HS: Highly significant; NS: Not significant



[Table/Fig-3]: Bar graph showing comparision of peroxide penetration between the Groups



[Table/Fig-4]: Line graph showing comparision of Groups 2-4, 4-6, 2-6

DISCUSSION

The experimental method selected in the current study has been described as an accurate and sensitive means to determine pulpal penetration of hydrogen peroxide [1,13]. This method is based on the oxidation reaction of leucocrystal violet buffer solution by hydrogen peroxide that is catalysed by horseradish peroxidise to produce a colour to demonstrate the presence of hydrogen peroxide [1].

Hydrogen peroxide breaks down into free radicals which eventually combine to form molecular oxygen and water. The oxygen oxidizes the stained area or the interprismatic organic matter, thus removing or lightening it. The effects of carbamide peroxide are similar to hydrogen peroxide because it ultimately breaks down into urea and water, with the latter then breaking down into free radicals, which eventually combine to form molecular oxygen and water [2-6].

Tooth enamel is the most dense part in the body, but studies have reported that both hydrogen peroxide and carbamide peroxide penetrate enamel and dentin. Subsequently, they enter the pulp chamber at various rates and the amount diffused is dependent on its original concentration, the length of time the agent is in contact with the dentin, the size and depth of the cavity, the type of base, bonding agent and restoration used [2,4,5,7,14-16].

The findings of this study shows that peroxide penetrates into the pulp of intact and restored teeth with higher pulpal peroxide penetration, that is statistically significant in restored teeth as compared to intact teeth. These findings are consistent with previous studies conducted [2,4,5]. The higher pulpal peroxide penetration amount in restored teeth is largely due to the microleakage properties of the restorative materials, as none of the materials can completely prevent microleakage [2,17].

This study also shows that pulpal peroxide levels in teeth restored with composite resins is lower than teeth restored with resin modified glass ionomer cement as various studies have already shown that composite resins show lower microleakage properties

than resin modified glass ionomer cement because of advanced adhesive technology [2,18].

The hazardous effects of hydrogen peroxide has been observed by various researchers [2,19-22]. However no serious problems associated with 10%carbamaide peroxide has been reported [3]. Clinical trials have also found sporadic and reversible reaction either for “in office” or “at home” techniques [23,24]. There have been some post-operative sensitivity reports because of reversible damage to the pulp after bleaching procedures [2,25-27].

This in vitro model is representative of the in vivo process, although, it is not known how closely it compares to the in vivo absorption of hydrogen peroxide in teeth with vital pulp during the bleaching processes. The pulp may protect itself from damage by hydrogen peroxide through enzymatic breakdown of the molecule by peroxidase and catalase. Pulp cells also produce haeme-oxygenase1, an important defensive enzyme produced at molecular level in respone to oxidative stress, specially found in odontoblasts and endothelial cells subjacent to the areas of bleached enamel. There are atleast two forces that might work against the diffusive flux of molecules of the bleaching agents towards the pulp: the positive pulpal pressure and osmotic pressure of the gels [5,7,19,28-30].

Although, the side-effects on the pulp are minimal and reversible, there is a need for caution in the use of bleaching agents, especially in restored teeth.

CONCLUSION

On the basis of these results and within the limitations of this in vitro study, it may be concluded that peroxide readily penetrates into the pulp through intact and restored teeth with restored teeth showing more peroxide penetration depending upon their microleakage properties, thus teeth restored with composite resin showed less peroxide penetration than teeth restored with resin modified glass ionomer cement.

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