Effect of bromelain enzyme on the microleakage of composite resin restorations after external tooth bleaching: An in vitro study

Aarti Mulgaonkar, Ida de Noronha de Ataide, Marina Fernandes, Rajan Lambor, Renita Soares
Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Bambolim, Goa, India

Abstract

Aims: The aim of this study is to evaluate in vitro the effect of application of bromelain enzyme on the microleakage of composite resin restorations after external tooth bleaching using spectrophotometric evaluation.

Subjects and Methods: Buccal Class V cavities were prepared on the surface of fifty intact premolars, which were randomly divided into five groups. All cavities were filled with composite resin.

• Group I: Teeth were not bleached but restored (n = 10). External bleaching with 35% hydrogen peroxide was carried out for the rest of the specimens
• Group II: Cavities were restored immediately after bleaching (n = 10)
• Group III: Cavities were restored after a delay of 3 weeks (n = 10)
• Group IV: Cavities were treated with sodium ascorbate after bleaching and then restored (n = 10)
• Group V: Cavities were treated with bromelain enzyme solution after bleaching and then restored (n = 10).

Microleakage was assessed by the dye extraction method using a spectrophotometer.

Statistical Analysis Used: The data were analyzed statistically by comparison of mean microleakage and post hoc test using SPSS 2.0 software.

Results: Group I displayed the least amount of microleakage, whereas Group II showed the greatest amount of microleakage (P < 0.05). Groups III, IV, and V showed a significantly lower amount of microleakage compared to Group II (P < 0.05). There was no statistically significant difference between Groups IV and V.

Conclusions: Microleakage increased significantly after external bleaching with 35% hydrogen peroxide, and decreased when the bleached teeth were treated with antioxidants. Ten percent bromelain enzyme was effective in decreasing microleakage; however, its efficacy was similar to 10% sodium ascorbate.

Keywords: Bromelain; composite; external bleaching; microleakage

INTRODUCTION

With the mounting demand for esthetics, vital tooth bleaching procedure has gained popularity over the last couple of decades.1,2 External tooth bleaching offers a fast, economical, and a conservative option for the treatment of discolored teeth.3 Different bleaching agents such as hydrogen peroxide and carbamide peroxide have been commonly used in different concentrations to attain a desirable result.1,4,5

The bleaching procedure may give rise to a number of complications, which may include postoperative sensitivity, pulpal irritation, tooth structure alterations, to
decreased bond strength of composite resin restorations to the enamel. Another important complication following bleaching is an increase in microleakage of composite restorations, which was extensively reviewed and reported in a systematic review by Attin et al.[7]

Bleaching agents mainly act through a complex oxidation reaction, which results in the release of oxygen and other free radicals.[8] An increase in microleakage of composite resin restorations associated with bleaching occurs mainly because of the presence of residual peroxide and residual oxygen. This tends to interfere with the adhesion of restorative materials by inhibiting resin polymerization.[9]

Different methods have been suggested to avoid this complication postbleaching, which may vary, including removal of the superficial enamel layer, use of adhesives containing organic solvents, pretreatment of enamel with alcohol, cleansing the cavity with antioxidants and a postbleaching period varying from 24 h to 3 weeks.[7]

The most commonly recommended method is to delay the bonding procedure until 2–3 weeks after bleaching.[10,11] Studies have shown that residual peroxide and oxygen dissipate over a period of time, eliminating any interference with bonding. However, this is a time-consuming method for both the practitioner as well the patient.[12]

Hence, the need for a quicker and convenient method lead to the utilization of several antioxidants such as sodium ascorbate, ascorbic acid, butylhydroxyanisole, catalase, ethanol, acetone, glutathione, peroxide, α-tocopherol, and sodium bicarbonate. According to research, only a few of these antioxidants were able to decrease the microleakage of composite resin restorations.[12] The interest in natural antioxidants of plant origin has greatly increased in recent years. Numerous natural antioxidants such as proanthocyanidins, lycopene have successfully shown to have a positive effect on bleached enamel.[13]

One such natural antioxidant is bromelain enzyme. It is a complex mixture of proteolytic enzymes derived from pineapple. It has a wide range of therapeutic benefits and has found applications in medicine, health, food, and cosmetics.[14]

To our knowledge, there is insufficient literature on microleakage of composite restorations following bleaching. Till date, no study has investigated the effect of bromelain enzyme on bleached enamel.

The aim of this study was to evaluate and compare the effect of bromelain enzyme on the microleakage of composite resin restoration after external tooth bleaching with 35% hydrogen peroxide.

**SUBJECTS AND METHODS**

**Preparation of antioxidant solution**

To prepare 10% sodium ascorbate solution 10 g of sodium ascorbate (Kemphasol, India) in the form of powder was dissolved in 100 ml of distilled water. Furthermore, 10 g of bromelain enzyme (Source naturals, USA) in the form of powder was collected from the capsule and dissolved in 100 ml of distilled water to make 10% bromelain enzyme solution.

**Sample preparation**

Fifty intact premolars that had been freshly extracted for orthodontic purposes were used. Teeth with caries, hypoplastic areas, or cracks and teeth that had a history of restorative/endodontic treatment were excluded. After extraction, the soft tissue, dental calculus, and stains were immediately removed from the surfaces of the teeth with the help of an ultrasonic scaler. Teeth were then stored in normal saline at 37°C until further use.

**Experimental groups**

The stored teeth were randomly divided into five experimental groups (n = 10 per group) after bleaching with 35% hydrogen peroxide (Pola office, SDI Limited, Bayswater, Victoria 3153, Australia) [Table 1]. Before bleaching, tooth surfaces were polished with oil- and fluoride-free fine pumice and water using a brush and a slow-speed hand-piece. All tooth surfaces were then rinsed and dried with an air syringe.

**Table 1: Distribution of specimens and study group**

| Groups   | Bleaching agent      | Antioxidants used                                      | Composite restoration                  |
|----------|----------------------|--------------------------------------------------------|----------------------------------------|
| Group I  | None                 | None                                                   | Done immediately                       |
| Group II | 35% hydrogen peroxide| None                                                   | Done immediately                       |
| Group III| 35% hydrogen peroxide| None                                                   | Done 3 weeks after bleaching           |
| Group IV | 35% hydrogen peroxide| 10% sodium ascorbate solution for 10 min               | Done immediately                       |
| Group V  | 35% hydrogen peroxide| 10% bromelain enzyme solution for 10 min               | Done immediately                       |

**Group I (unbleached/control group)**

Group I teeth underwent cavity preparation and restoration but were not bleached. Box-shaped Class V cavities were made on the buccal surface, around the cementoenamel junction, using a round diamond bur in high-speed hand-piece under air and water-cooling. Occlusal margins and gingival margins of the prepared cavities were located in the enamel and the root, respectively. The prepared...
cavities were approximately 2 mm in height, 3 mm in the mesiodistal direction, and 2 mm in depth. The samples were etched with 37% phosphoric acid for 15 s, rinsed with water for 20 s, and bonded, followed by composite buildup. Following this, composite resin (Tetric N-Ceram, shade A3, Ivoclar Vivadent) was placed into cavities and polymerized in 1.5–2 mm-thick layers for 20 s. The composite resin was then finished with fine-grit finishing bur, and the teeth were preserved at 37°C in artificial saliva (E saliva, Entod Pharma Ltd. India) that was changed daily for 10 days. The same operator carried out the standardized procedure.

**Group 2 (immediate restoration group)**
In Group 2, the teeth were first bleached by the following method and then immediately restored with composite resin. The teeth were bleached with 35% hydrogen peroxide, Pola Office (SDI Limited, Bayswater, Victoria 3153, Australia) with three applications of 8 min each. Following bleaching, the specimens were thoroughly rinsed with water for 1 min. The bleached teeth were stored in fresh artificial saliva at 37°C between treatments. After the final bleaching, the cavities were immediately prepared and restored as described above for Group 1.

**Group 3 (delayed restoration group)**
In Group 3, the teeth were bleached as described above and then, after the final rinse, immersed in artificial saliva at 37°C. After 3 weeks, cavities were prepared, and composite resin restorations were placed as in Group 1.

**Group 4 (sodium ascorbate treated group)**
In Group 4, the teeth were bleached, and the cavity prepared immediately as described above for Group 2. The specimens were exposed to 10% sodium ascorbate for 10 min using a cotton pellet before filling with composite resin. After removal of the cotton pellet, the cavity was rinsed with distilled water for 2 min and dried gently with air to prepare it for composite resin restoration. Composite resin restorations were placed as in Group 1.

**Group 5 (bromelain enzyme-treated group)**
After bleaching, a novel antioxidant agent, 10% bromelain enzyme was applied to cavities in the bleached teeth, as described above for Group 4. The samples were restored, as described in Group 1.

**Microleakage test (using dye extraction method)**
All surfaces of the teeth, except for a 1-mm zone surrounding the restoration margins, were covered with three coats of nail varnish, the apices of the roots were sealed with sticky wax and immersed in 2% methylene blue solution for 24 h. After 24 h, the samples were rinsed under tap water for 30 min, and nail varnish was removed using an ultrasonic scaler. Then, the samples were placed in vials containing 65 wt% nitric acid for 3 days in centrifugal tubes. The vials were centrifuged at 14,000 rpm for 5 min, and following that, the supernatant solutions thus collected were used to determine absorbency in an ultraviolet (UV) visible spectrophotometer at 550 nm using concentrated nitric acid as the blank. The results were recorded as a measure of transmission of light. The data were analyzed statistically by comparison of mean microleakage and post hoc test using SPSS 2.0 software (Spss Inc., Chicago, IL, USA).

**RESULTS**
The results are presented in Table 2:
- Mean average microleakage of the control Group 1 (0.026 ± 0.001) was significantly lower than all other groups except Group III (delayed restoration group) (0.031 ± 0.003) ($p < 0.05$)
- Group II (immediate bonding) had the highest value (0.066 ± 0.005) among all the groups.
- Among the experimental groups, Group IV (0.038 ± 0.006) and Group V (0.038 ± 0.003) showed significantly lower microleakage values as compared to Groups II and III. However, there was no statistically significant difference between the two.

**DISCUSSION**
Different variables affect the longevity of direct restorations, which could be clinician related, patient related or restoration related. The ability of restoration to bond to tooth structure helps achieve a stronger and durable restoration. Failure of composite restorations may be attributed to numerous factors such as polymerization shrinkage, secondary caries, fracture of the restoration, and microleakage at the tooth-restoration interface. Various research articles have reported diminished bond strength of the composite restorations to enamel following bleaching. However, to date, relatively few studies have researched on the increase in the microleakage of composite resin restorations following tooth bleaching, specifically external tooth bleaching.

The present study demonstrated that external bleaching with 35% hydrogen peroxide (Groups II–V) significantly

### Table 2: Descriptive statistics showing mean, standard deviation of microleakage for all the groups

|      | n | Mean   | SD    |
|------|---|--------|-------|
| Group 1 | 10 | 0.02620 | 0.00103 |
| Group 2 | 10 | 0.06670 | 0.005851 |
| Group 3 | 10 | 0.03180 | 0.003676 |
| Group 4 | 10 | 0.03860 | 0.006293 |
| Group 5 | 10 | 0.03880 | 0.003706 |

SD: Standard deviation
increased the microleakage values of restorations compared to the unbleached group (Group I) [Table 3], which was in accordance with a study done by Han et al.\textsuperscript{[12]} The presence of residual peroxide and oxygen postbleaching is the main reason for this increase in microleakage.\textsuperscript{[7–9]} Titley et al.\textsuperscript{[22]} suggested that the dentin and dentinal fluid acts as a free radical reservoir, which interferes with resin attachment and inhibits resin polymerization.

Dishman et al.\textsuperscript{[23]} noted that resin tags in bleached enamel were short, sparse, poorly defined, structurally incomplete, or completely absent when restored. This explains the results displayed by Group II (immediate restoration) wherein the microleakage was the highest.

The peroxide ions decompose or are released through surface diffusion upon storage for 2–3 weeks, thereby reversing the structural changes caused by bleaching.\textsuperscript{[24]} This particular finding was supported by the result obtained in Group III (delayed restoration) where the microleakage value had decreased and was similar to Group I (unbleached group). Thus, concluding that composite restorations done after a period of 2–3 weeks eventually eliminates any reduction in microleakage associated with bleaching. However, this is time-consuming for both the practitioner and the patient.\textsuperscript{[12]}

Hence a quick and effective method to reverse the effect of residual free radicals is the application of antioxidants to the cavity. Therefore, this study is aimed to investigate the effect of a new antioxidant (Bromelain enzyme) on the microleakage of composite resin restorations after external tooth bleaching with 35% hydrogen peroxide.

In the present research, the authors noted significant differences \( (P < 0.05) \) when comparing microleakage in the unbleached control group to that of groups that had antioxidant treatment (Groups IV and V) [Table 4]. As reported by Han et al.\textsuperscript{[12]} this suggests that antioxidants play an important role in decreasing microleakage when restorations are done immediately after bleaching.

Moreover, in the present study, except for the unbleached group, the lowest microleakage values were observed in samples treated with antioxidants, such as sodium ascorbate (Group IV), or with bromelain enzyme (Group V).

However, there was no statistically significant difference between Group IV (sodium ascorbate) and Group V (bromelain enzyme).

Sodium ascorbate is a potent antioxidant capable of quenching the reactive free radicals. It neutralizes the effect of the residual oxygen layer, allows free radical polymerization of resin base materials by restoring the altered redox potential of the oxidized bonding substrate, thus reversing the compromised bonding.

The utilization of plant extracts as a viable alternative to chemical and synthetic antioxidant have been encouraging.\textsuperscript{[13]} Bromelain enzyme is still a recent entity in restorative dentistry, and as yet, there has been only a single study that has been conducted to test its deproteinizing properties.\textsuperscript{[25]} In the current study, the authors researched on its ability to function as an antioxidant. The antioxidant property of bromelain enzyme may be from the amino acid content, which can scavenge the radical by donation of the hydrogen atom.\textsuperscript{[14]}

35% hydrogen peroxide (Pola office) was used for three applications of 8 min each. The reason for the gel to be refreshed is due to the fast degradation of peroxide. Studies have shown that hydrogen peroxide used for tooth whitening has a pH around 7 immediately after application, but when placed for a longer period, the pH of the gel decreases to 5, which may increase tooth sensitivity.\textsuperscript{[26]}

In the present study, the results were recorded as a measure of absorbance of light. According to Beer–Lambert’s law,
of microleakage as that of sodium ascorbate.

CONCLUSIONS

- Microleakage increased significantly after external bleaching with 35% hydrogen peroxide and decreased when teeth were treated with antioxidant
- Bromelain enzyme had similar efficacy in reducing microleakage as that of sodium ascorbate.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Vidhya S, Srinivasulu S, Sujatha M, Mahalaxmi S. Effect of grape seed extract on the bond strength of bleached enamel. Oper Dent 2011;36:453-8.
2. Carlos RG, Kand FA, Alessandra BB. The effects of antioxidant agents as neutralizers of bleaching agents on enamel bond strength. Braz J Oral Sci 2006;5:971-6.
3. Kimyai S, Oskoei SS, Rafighi A, Valizadesh H, Ajami AA, Heilali ZN. Comparison of the effect of hydrogel and solution forms of sodium ascorbate on orthodontic bracket-enamel shear bond strength immediately after bleaching: An in vitro study. Indian J Dent Res 2010;21:54-8.
4. Bulut H, Kaya AD, Turkmun M. Tensile bond strength of brackets after antioxidant treatment on bleached teeth. Eur J Orthod 2005;27:466-71.
5. Turkmen M, Celik EU, Kaya AD, Ariol M. Can the hydrogel form of sodium ascorbate be used to reverse compromised bond strength after bleaching? J Adhes Dent 2009;11:38-40.
6. Gokce B, Cemlekooglu ME, Ozpinar B, Turkmen M, Kaya AD. Effect of antioxidant treatment on bond strength of a luting resin to bleached enamel. J Dent 2008;36:780-5.
7. Attin T, Hannig C, Wiegang A, Attin R. Effect of bleaching on restorative materials and restorations - A systematic review. Dent Mater 2004;20:852-61.
8. Turkmen M, Kaya AD. Effect of 10% sodium ascorbate on the shear bond strength of composite resin to bleached bovine enamel. J Oral Rehabil 2004;31:1184-91.
9. Kaya AD, Turkmen M, Arici M. Reversal of compromised bonding in bleached enamel using antioxidant gel. Oper Dent 2008;33:441-7.
10. Bittencourt ME, Trentin MS, Linden MS, de Oliveira Lima Arsati YB, Fraça FM, Flório FM, et al. Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations. J Am Dent Assoc 2010;141:300-6.
11. Da Silva Machado J, Cándido MS, Sundfeld RH, de Alexandre RS, Cardoso JD, Sundfeld ML. The influence of time interval between bleaching and enamel bonding. J Esthet Restor Dent 2007;19:111-9.
12. Han Y, Mo S, Jiang L, Zhu Y. Effects of antioxidants on the microleakage of composite resin restorations after external tooth bleaching. Eur J Dent 2014;8:147-53.
13. Subramoniam R, Mathai V, Christaine Angelo JB, Ravi J. Effect of three different antioxidants on the shear bond strength of composite resin to bleached enamel: An in vitro study. J Conserv Dent 2015;18:144-8.
14. Maurer H. Bromelain: Biochemistry, pharmacology and medical use. Cell Mol Life Sci 2001;58:1234-45.
15. Fernandes NA, Vally ZL, Skyes LM. The longevity of restorations: A literature review. S Afr Dent J 2015;70:410-3.
16. Demarco FF, Corrêa MB, Cenci MS, Moraes RR, Opdam NJ. Longevity of posterior composite restorations: Not only a matter of materials. Dent Mater J 2012;28:87-101.
17. Sasaki RT, Flório FM, Basting RT. Effect of 10% sodium ascorbate and 10% alpha-tocopherol in different formulations on the shear bond strength of enamel and dentin submitted to a home-use bleaching treatment. Oper Dent 2009;34:746-52.
18. Torres CR, Koga AF, Borges AB. The effects of anti-oxidant agents as neutralizers of bleaching agents on enamel bond strength. Braz J Oral Sci 2006;5:971-6.
19. Lai SC, Tay FR, Cheung GS, Mak YF, Carvalho RM, Wei SH, et al. Reversal of compromised bonding in bleached enamel. J Dent Res 2002;81:477-81.
20. Bulut H, Turkun M, Kaya AD. Effect of an antioxidant agent on the shear bond strength of brackets bonded to bleached human enamel. Am J Orthod Dentofacial Orthop 2006;129:266-72.
21. Kum KY, Lim KR, Lee CY, Park KH, Safavi KE, Fouda AF, et al. Effects of removing residual peroxide and other oxygen radicals on the shear bond strength and failure modes at resin-tooth interface after tooth bleaching. Am J Dent 2004;17:267-70.
22. Tittley KC, Torneck CD, Smith DC, Chernockey R, Adibfar A. Scanning electron microscopy observations on the penetration and structure of resin tags in bleached and unbleached bovine enamel. J Endod 1991;17:72-5.
23. Dishman MV, Covey DA, Baughan LW. The effects of peroxide bleaching on composite to enamel bond strength. Dent Mater 1994;10:33-6.
24. Arumugam MT, Nesamani R, Kitappa K, Sanjeev K, Sekar M. Effect of various antioxidants on the shear bond strength of composite resin to bleached enamel: An in vitro study. J Conserv Dent 2014;17:22-6.
25. Dayem RN, Tameesh MA. A new concept in hybridization: Bromelain enzyme for deproteinizing dentin before application of adhesive system. Contemp Clin Dent 2013;4:421-6.
26. Reis A, Tay LY, Herrera DR, Kossatz S, Loguercio AD. Clinical effects of prolonged application time of an in-office bleaching gel. Oper Dent 2011;36:590-6.
27. Measurements A. Spectrophotometry. Chem 111 lab 2005;21:1-8.
28. Camps J, Pashley D. Reliability of the dye penetration studies. J Endod 2003;29:592-4.
29. Veríssimo DM, do Vale MS. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: A critical review. J Oral Sci 2006;48:93-8.