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Comparative Evaluation of Effect of Three Different Antioxidants on Post-Bleaching Microleakage of Composite Resin Restoration – An in Vitro Study

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Abstract

Introduction: Composite restoration immediately after teeth bleaching has shown increased microleakage. Recent studies suggested that antioxidant application reduces amount of microleakage.

Aim: To evaluate and compare the effect of 3 different antioxidants on postbleaching microleakage of composite resin restoration.

Method: 20 mandibular premolar teeth were selected followed by bleaching procedure. Teeth were alloted randomly into 4 groups.

Group 1 - no antioxidant application on 5 teeth.

Group 2 – 5 teeth bleached and Proanthocynidin antioxidant application,

Group 3 – 5 teeth bleached and Bromelain antioxidant application,

Group 4 – 5 teeth bleached and Quercetin antioxidant application,

For 10 minutes. Surfaces rinsed followed by immediate composite restoration. Teeth were sectioned and microleakage was examined using silver nitrate dye with stereomicroscope under 20x magnification.

Statistical Analysis: The data were analyzed with the Kruskal–Wallis's test to evaluate the differences in the microleakage of tested groups. Mann–Whitney statistical test was used to compare the groups in pairs.

Result: Group 2 showed the minimum amount of microleakage, whereas Group 1 showed the maximum amount of microleakage ($P < 0.05$). There was no statistically significant difference between Groups 3 and 4.

Conclusion: Microleakage decreased significantly after external bleaching followed by application of antioxidants.

Keywords: Antioxidants, Bromelain, Microleakage, Proanthocynidin, Quercetin, Silver Nitrate Dye, Vital Bleaching Procedure.

Introduction

With the increasing demand for esthetics, vital tooth bleaching procedure has gained popularity over the last couple of decades[1,2]. Discoloured teeth should be treated by external tooth bleaching which offers a fast, economical and a conservative option[2]. Hydrogen peroxide has a clear liquid form and is highly unstable, odorless and acidic. Gaseous bubbles are produced by oxidation reactions of peroxides under the enamel surface. Strength of enamel–resin bonds is affected by the oxygen-containing surface layer that inhibits the polymerization of bonding agents[4,5]. Various studies suggest suspending the restoration procedure for 2–4 weeks to prevent bond failure between enamel and composite restorations[20] However, this causes a clinical problem when the tooth should be immediately restored with a composite restoration. Therefore, a material that can accelerate the loss of peroxide residue is required[6].

Several natural antioxidants such as proanthocyanidins, lycopene have successfully shown to have a positive effect on bleached enamel[2].

Proanthocyanidins (PACs) are non-enzymatic natural antioxidants having large molecular size, which can limit its ability to penetrate into the demineralised collagen matrix [3,4,7,8,10,11,15].

Bromelain is enzyme based antioxidant having complex mixture of proteolytic enzymes derived from pineapple. It is a protein, which functions as an enzyme and belongs to a subclass of enzymes known as proteolytic enzymes (proteases). The function of proteases is to catalyze the hydrolysis of proteins to give amino acids[5,6,10].

Another antioxidant material is quercetin which is a flavonoid available in many vegetables, fruits, and foods. It is also available as food supplements and can be potentially used abundantly for reversing the effect of bleaching on Shear Bond Strength.[7,8,9,14].

Studies suggest that bleaching procedures may adversely affect the physical properties, color, and marginal integrity of restorative materials and also the bonding of enamel and dentin. Microleakage due to frequent composite restorations before and after bleaching is another side effect of teeth bleaching materials.

The dye penetration technique is one of the most used methods for assessing marginal seal[1,13]. Considering the penetration capacity of the silver nitrate solution, its use is considered a very severe test because the diameter of

the silver ion is very small (0.059 nm) when compared to the mean size of a bacteria (0.5-1.0 μm)[12,13,14].

To our knowledge, there is insufficient literature on microleakage of composite restorations immediately after bleaching. Till date, no study has compared the effect of Proanthocynidin, bromelain and Quercetin on bleached enamel.

Thus, the aim of the study was to evaluate and compare the effect of 3 different antioxidants on postbleaching microleakage of composite resin restoration.

Subjects and method

Preparation of antioxidants

To prepare 5% Proanthocynidin solution 5 g of Proanthocynidin (Kemphasol, India) in the form of powder was dissolved in 100 ml of distilled water.

Furthermore, 5 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 5% bromelain enzyme solution.

To prepare 1% Quercetin, 1g Quercetin powder mixed with approx.5ml of ethanol and 100ml of distilled water.

Sample Preparation

Inclusion criteria

- Teeth extracted for orthodontic purposes

Exclusion criteria

- Teeth with – carious lesion
- Hypoplastic areas or cracks
- Teeth that had a history of restorative/endodontic treatment

20 freshly extracted intact premolars were selected. Ultrasonic scaler was used to remove dental calculus and stains from the surfaces of the teeth. Teeth were then stored in normal saline at 37°C until further use.

Experimental Groups

Bleaching procedure with 35% hydrogen peroxide (Pola office, SDI Limited, Bayswater, Victoria 3153, Australia)was performed in all 20 teeth. They were randomly divided into four experimental groups as shown in Table 1 (n = 5 per group)

Group 1 (N=5) -Teeth underwent cavity preparation and restoration after bleaching. Box-shaped Class V cavities were made on the buccal surface, around the cementoenamel junction, using a round diamond bur with 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 2.5 mm in height, 4.5 mm in the mesiodistal direction, and 1 mm in depth(fig.1). The samples were bonded with 7th generation bonding agent(Clearfil SE,Kuraray) for 10 seconds curing, followed by composite buildup with Filtek Z-250 (3M ESPE, St. Paul, MN, USA) and polymerized in 1.5–2 mm-thick layers for 20 s.

Group 2 (N=5) – Teeth were bleached and exposed to 5 % proanthocynidin for 10 min using a cotton pellet and rinsed with water. The cavities were prepared and restored as described in Group 1.

Group 3 (N=5) –Teeth were bleached and exposed to 5 % breomelain for 10 min using a cotton pellet and rinsed with water. The cavities were prepared and restored as described in Group 1.

Group 4 (N=5) – Teeth were bleached and exposed to 1 % quercetin for 10 min using a cotton pellet and rinsed with water. The cavities were prepared and restored as described in Group 1.

Microleakage test

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.

After immersion in the silver nitrate solutions for 24 h, the specimens were washed, immersed in a photographic

developer (Eastman; Kodak Co, Rochester, NY, USA) for 8 h under fluorescent light, and then abundantly washed under running water.

Afterwards, the specimens were sectioned parallel to the long axis buccolingually with a low-speed water-cooled thin sectioning diamond machine (Gillings-Ham Co., NY, USA). These samples were evaluated under a stereo-microscope (Zeiss, Germany) at 30X magnification to examine their microleakage. The results were checked by two independent examiners who were blinded to the study groupings. Next, each examiner reported the depth of the dye penetration based on the following scale:

Microleakage Evaluation Scale (fig.2)

- 0=No Microleakage(fig.2a)
- 1=Microleakage penetrated to ½ depth of the cavity wall(fig.2b)
- 2=Microleakage penetrated beyond the ½ depth of the cavity wall(fig.2c)
- 3=The microleakage penetration reached the cavity bottom surface(fig.2d)

Statistical analyses

The data were analyzed with the Kruskal–Wallis's test to evaluate the differences in the microleakage of the tested groups. Mann–Whitney statistical test was used to compare the groups in pairs. The Stata software, version 11 was used for the purpose of data analysis. The statistical significance level was set at P<0.05.

Results

Kruskal Wallis test; * indicates significant difference at p≤0.05

The difference in microleakage among four groups was statistically significant. Group 2<Group 4<Group 3<Group 1(table 2)

Mann whitney test; * indicates significant difference at p≤0.05

Mean average microleakage of the control Group 1 (17.6) was significantly higher than all other groups (P < 0.05)

Group 2. (Immediate bonding) had the lowest value (5.20) among all the groups

Group 4. (8.50) and Group 3 (10.70) showed significantly lower microleakage values as compared to Groups 1. However, there was no statistically significant difference between the two.

Discussion

Numerous studies have been conducted on the interactions of bleaching materials and the bonding strength of composite resin to the bleached teeth enamel[21]. These studies showed a significant reduction in the bonding strength of composite resin to the bleached enamel compared to the unbleached ones. 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[22]. The results of the current study demonstrated a significantly higher increase in microleakage into the enamel and dentin layers in the groups that had immediate composite restoration following teeth bleaching than those observed in the non-bleached groups. Our findings were consistent with those reported by previous studies.

Two previous studies[1,2]have demonstrated negative effects of composite contact with 35% hydrogen peroxide and marginal seal. Further, Shinohara, et al. 25 have shown that immediate dental filling after bleaching of teeth has contributed to higher microleakage into dentin margins.

A previous study conducted by Al-Hasani, et al. [23]has also emphasized that a 7-14 days delay for avoiding the negative effect of bleaching on the microleakage of filling materials. There was a significant reduction of the

microleakage of three antioxidants (sodium ascorbate, ascorbic acid and vitamin C).

In present study, proanthocynidin showed lesser amount of microleakage as compared to bromelain and quercetin because i) it has specificity for hydroxyl free radicals that entraps free radicals (ROS) produced during bleaching procedure which prevents interference in formation of resin tags thereby increasing bonding ability and decreasing microleakage ii) it has multiple electron donor sites, 50 times more than ascorbic acid (Vit C) and 20 times more than olive oil (Vit E), thus within less time and more efficiently it binds to free radicals and helps in reducing microleakage.[1,3,4,7,8,10,11,15]

Quercetin showed lesser microleakage than bromelain; but difference was non-significant a flavonoid has i) small molecular size which penetrates deeper in dentinal tubules ii) It has affinity to bond proline rich protein, forms hydrogen bond with collagen fibrils. Various studies have suggested that it increases modulus of elasticity, tensile strength and other mechanical properties[7,8]. iii) it inhibits (ROS) thereby reducing production of COX2 and MMPs which inturn reduces inflammatory action. Thus, there are less chances of cervical resorption when applied quercetin after bleaching.

Bromelain(Group C) showed lesser amount of microleakage than control group(Group A)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-removes unsupported collagen in tooth[22]. 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[13]it increases dentin permeability that increases monomer diffusion that causes deacrese in microleakage[2,5].

The future scope for *in vitro* studies should include utilizing an experimental antioxidant formulation such as above three described in this article includes it should be determined whether the higher microleakage rates that were observed *in vitro* subsequent to bleaching translate to significant deterioration of restorations in actual clinical situations and whether this deterioration can be reduced by using an effective antioxidant formulation. In addition, determining the minimum time required for antioxidant treatment after external tooth bleaching is necessary.

Conclusion

1. Microleakage decreased significantly after external bleaching followed by application of antioxidants.
2. Newly introduced antioxidants Quercetin & Bromelain showed relatively equal efficacy in decreasing microleakage as that of Proanthocynidin, thus can be use as an alternative.

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Legend of Tables and Figures

Table 1: Antioxidant application followed by composte restoration of bleached teeth

Groups	Bleaching agent	Antioxidants used	Composite restoration
Group 1	35% hydrogen peroxide	None	Done immediately
Group 2	35% hydrogen peroxide	5% Proanthocynidin solution for 10 min	Done immediately
Group 3	35% hydrogen peroxide	5% Bromelain enzyme solution for 10 min	Done immediately
Group 4	35% hydrogen peroxide	1% Quercetin solution for 10 min	Done immediately

Table 2: Comparison of microleakage among four groups

Group		Score 0	Score 1	Score 2	Score 3	Total	Mean rank	p value
Group 1	N	0	0	1	4	5	17.60	0.006*
	%	0.00%	0.00%	20.00%	80.00%	100.00%		
Group 2	N	3	2	0	0	5	5.20	
	%	60.00%	40.00%	0.00%	0.00%	100.00%		
Group 3	N	1	1	3	0	5	10.70	
	%	20.00%	20.00%	60.00%	0.00%	100.00%		
Group 4	N	1	3	1	0	5	8.50	
	%	20.00%	60.00%	20.00%	0.00%	100.00%		

Figure 1: Class 5 Cavity Preparation

Figure 2: Stereomicroscopic images at 30x magnification. Microleakage evaluation scale 2a: Score, 2b:Score 1, 2c: Score 2, 2d: Score 3