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Effect of Chitosan Nanoparticle as an Antioxidant Material on Shear Bond Strength of Composite Resin to Enamel after External Bleaching

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Abstract

BACKGROUND: If composite resin restoration is required following external bleaching treatment, a waiting period of between 1 and 3 weeks is needed to avoid restoration failure. However, patients usually need faster treatment.

AIM: This study aimed to investigate the effect of chitosan nanoparticles as an antioxidant material on the shear bond strength (SBS) of composite resin restorations to enamel after external bleaching.

METHODS: Thirty premolars were used in this study and randomly assigned into four groups of six samples, Group 1, no bleaching – no antioxidant, Group 2, bleaching – composite restoration, Group 3, bleaching – waiting 2 weeks – composite restoration, Group 4, bleaching – sodium ascorbate – composite restoration, Group 5, bleaching – chitosan nanoparticles – composite restoration. The SBS was tested with a universal testing machine. One-way analysis of variance and the Turkey's test were used for statistical data analysis ($p < 0.05$).

RESULTS: The group that applied chitosan nanoparticles to the teeth previously bleached and then restored with composite resin resulted in similar SBS as the other groups ($p > 0.05$), except for the group that was bleached and directly restored with composite resin ($p = 0.000$).

CONCLUSION: The chitosan nanoparticle has the potential as an antioxidant material since it produces similar SBS to composite resin restorations on enamel after external bleaching than the other groups.

Introduction

Bleaching treatment has been very popular in the community because it can improve the esthetics of discolored anterior teeth. The mechanism of bleaching agents in whitening teeth is based on oxidation-reduction reactions, which release free radicals such as hydroxyl, superoxide anion, nascent oxygen, and perhydroxyl [1]. Free radicals are molecules that have unpaired electrons; hence, they are very reactive [2]. This molecule can react with the electron-rich pigment portion of the tooth structure, thereby breaking large, pigmented molecules into smaller molecules, resulting in reduced discoloration. These free radicals can penetrate the tooth structure through the enamel prisms, which can then reach the dentine [3].

Previous studies reported that bleaching agents such as hydrogen peroxide with a concentration of 30–40% and carbamide peroxide with a concentration of 10–15% could reduce the shear bond strength (SBS) of composite resin restoration to the enamel if the bleached enamel directly applied to the composite resin [4]. This condition is induced by the presence of free radicals, such as oxygen residues originating from bleaching agents, which can intervene in the adhesive agent polymerization, thereby damaging the bond strength between the composite resin and the tooth structure [5].

Preceding investigators have shown that residual oxygen will disappear over time, and composite resin can adhere to enamel that has been bleached [6]. Therefore, it is recommended to delay performing composite resin restorations to avoid the above conditions [7]. Moreover, the recommended delay time is between 24 h and 3 weeks [8]. The recommendation for delaying this restoration is based on a decrease in the adhesive strength of the composite resin on enamel or dentine after bleaching treatment, and this decrease in strength is only a temporary condition [9]. However, in clinical conditions, patients sometimes need fast treatment for certain reasons; thus, delaying restoration is not possible. According to Sharafeddin and Farshad [6], delaying composite resin restorations for 2 weeks showed an upsurge in the SBS of the restoration to the enamel that was bleached, and its SBS was equivalent to the SBS of restorative material to teeth that were not bleached. Several studies recommend the use of antioxidant material to speed up treatment. The antioxidant material that is often used is sodium ascorbate. Sodium ascorbate with an application time of 10 min was revealed to increase the SBS of composite resins [10], [11].

Finally, chitosan has been commonly employed in the health sector because of its plenty of advantageous attributes. Chitosan is a natural polysaccharide created from the deacetylation of chitin, which is obtained from crustacean marine animals' shells such as crabs and shrimp. Chitosan is abundant, cheap, and non-toxic. Previous research explained that chitosan has antioxidant properties [12], [13]. Another study by Mohamed *et al.* [14] showed that the application of 0.2% chitosan before restoration with composite resin resulted in a higher microtensile strength than 2.5% chitosan. Antioxidant attributes are exhibited by their capability to prevent reactive oxygen species (ROS) and lipid oxidation in biological systems. Chitosan can also capture free radicals from hydrogen donations or free electron pairs. The hydroxyl (OH) and amino (NH_2) groups in chitosan behave as antioxidants [15].

Chitosan in the form of nanoparticles has better absorption as an antioxidant than chitosan with regular sizes [16]. Modification of the physical form of chitosan into nanoparticles is carried out to optimize the effectiveness of chitosan because it has better absorption and penetration power than that which is not in the form of nanoparticles [17].

Adequate physical properties of a direct restoration material, one of which can be seen from its ability to produce permanent SBS between the tooth tissue and the restorative material [18]. The SBS test is widely utilized to test the bond strength of restorative material to enamel. In addition, this testing is simple, easy to perform, and accurate [19].

However, until now, there has been no study about the effect of chitosan nanoparticles as an antioxidant material on the SBS of composite resin restorations after external bleaching. Thus, this study aimed to investigate the effect of chitosan nanoparticles as an antioxidant material on the SBS of composite resin restorations to enamel after external bleaching. The null hypothesis of this study is that chitosan nanoparticle does not influence the SBS of composite resin restoration to enamel after external bleaching.

Materials and Methods

The research protocol has been approved by the Institutional Ethics Committee Faculty of Dentistry, Universitas Gadjah Mada under the number 083/KE/FKG-UGM/EC/2022 and conducted in accordance with the Declaration of Helsinki. A total of 30 premolars without caries, fractures, cracks, and no previous restorations were used in the study. The teeth roots were cut 2 mm below the cementoenamel junction using a microtome (diamond saw). The crown of each tooth was cut in the mesiodistal direction; thereby, the part of the tooth used was the buccal enamel surface. Each tooth was embedded in a self-curing acrylic resin mold (a length of 2 cm, a width of 2 cm, and a height of 2 cm) with the buccal surface facing up. The enamel surface was roughened and polished with 600-grit silicon carbide paper (Moyco, Montgomeryville, PA, USA) to attain a flat and rough enamel surface that was even and coarse. Samples were viewed with a light microscope to ensure that only the enamel and not the dentine surface were exposed.

Preparation of antioxidant material

The solution consisting of sodium ascorbate (10%) was made by diluting sodium ascorbate in distilled water at room temperature. The carbomer (Carpobol gel 934, Noveon, Brussels, Belgium with 2.5% [wt/wt]) was mixed with sodium ascorbate by diffusing the carbopol resin in distilled water. The blend was agitated until thick and then counteracted by adding dropwise triethanolamine to accomplish transparent gel. The amount of triethanolamine was adjusted to attain a hydrogel with a pH of 7 [20]. The 0.2% chitosan nanoparticle hydrogel was produced by diluting 0.2 g of chitosan powder (PT NHI, Serpong, Tangerang, Indonesia). Chitosan nanoparticle was already in hydrogel form.

Sample preparation

Thirty premolars were then randomly divided into five groups, each comprising six samples. Group 1, no bleaching and no application of antioxidant material, samples were soaked in artificial saliva for 2 weeks (served as a control). Group 2, the samples were bleached with 40% hydrogen peroxide (Opalescence Xtra Boost 40%, Ultradent, South Jourdan, UT, USA) according to the company's instructions (twice applications, each application of bleaching material for 30 min, between the two applications, and the samples were cleaned with distilled water before the second application of bleaching material). After bleaching, the composite resin restoration was immediately carried out. The enamel of the buccal surface was applied with 37% phosphoric acid (SDI Super Etch, SDI, Vic, AU) for 20 s, washed with water for 30 s, and gently air dried for 10 s. A fifth generation of adhesive material (Aper Single Bond2 Adhesive, 3M ESPE Dental Products, St. Paul, MN, USA) was applied, mildly spread with an air syringe, and polymerized using a light cure for 10 s. The implanted samples were equipped in a device having a split metal mold with a round cavity (diameter = 2 mm and height = 4 mm). A bulk of composite resin (Filtek Supreme, 3M ESPE Dental Products) was condensed into the cavity of the split mold and polymerized for 20 s. As a result, the composite resin is attached to the buccal segment of the crown.

In Group 3, samples were bleached with 40% hydrogen peroxide, according to Group 2, then soaked in artificial saliva for 2 weeks, and then composite resin restoration was carried out. In Group 4, the samples were bleached with 40% hydrogen peroxide, similar to Group 2; after bleaching, the samples were immediately applied with 10% sodium ascorbate as an antioxidant. In Group 5, the samples were bleached with 40% hydrogen peroxide and the same procedure as Group 4; however, the samples were applied with 0.2% chitosan nanoparticles. A syringe was employed to apply each antioxidant to each sample, and 0.02 mL of antioxidants were placed and dispersed onto the enamel surface using a sponge pellet for 10 min. The enamel surface was then cleaned using distilled water for 30 s. After applying antioxidants, Groups 4 and 5 were restored using composite resin as Group 2. All samples were immersed in artificial saliva and stored for 24 h in an incubator (37°C) and subjected to 500 thermocycles between 5° and 55°C in distilled water.

SBS test

Each sample was installed into the universal testing machine (Pearson Parke Equipment Ltd., London, UK) for testing SBS. The long axis of the sample was vertical to the way of the related forces. The knife edge was inserted at the edge between the composite resin restoration and the enamel surface. The SBS was assessed in a shear manner at a crosshead speed of 1 mm/min until a breakage appeared. The greatest force applied to the sample at the time of breakage was documented in Newton. To convey the SBS in megapascals, the documented value was divided by the cross-sectional area of the sample (mm^2). The fracture sites between the enamel and the composite resin were then observed using scanning electron microscopy (SEM) (JSM-IT200, Akishima, Tokyo, Japan) at a magnification of ×300. Failure types are categorized into three, namely: (1) adhesive; no signs of enamel fracture or residual composite resin on the teeth; (2) cohesive: Fracture of enamel or composite resin; and (3) A mix of adhesive and cohesive [17].

Statistical analysis

Data obtained showed normal (Shapiro–Wilk) and homogeneous (Lavene's test) distributions and were statistically analyzed using one-way analysis of variance (ANOVA) and Turkey's test. The IBM SPSS Statistics Software, version 23 (IBM Corp., Armonk, NY, United States) was used to process and analyze the data. The significance level was 95%.

Results

The one-way ANOVA analysis showed that chitosan nanoparticles affected the SBS of composite resin restorations to enamel after external bleaching treatment ($p = 0.000$). It can be seen in Figure 1 that samples were restored using composite resin directly after the bleaching treatment had the lowest SBS compared to other groups ($p = 0.000$). On the contrary, Group 1 (control), which was un-bleached, generated the highest SBS compared to other groups ($p < 0.05$), but this SBS value was nearly the same as Group 5, which was bleached and applied antioxidant material with chitosan nanoparticle ($p = 0.195$). The group with the 2-week waiting time before composite resin restoration (Group 3) produced the statistically same value as antioxidant groups (Groups 4 and 5) ($p > 0.05$). Antioxidant material using chitosan nanoparticles generated a similar value of SBS to sodium ascorbate ($p = 0.124$).

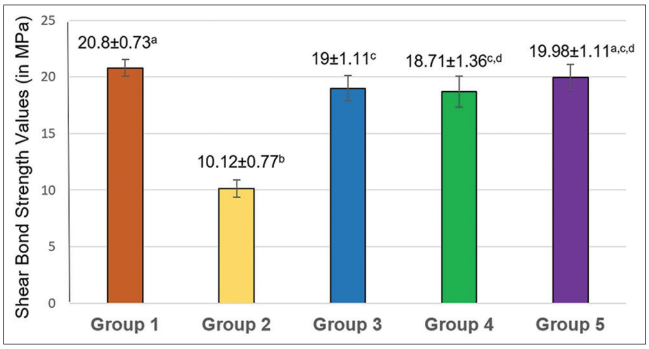

Figure 1: Shear bond strength values (in MPa). Different letters indicate that there were statistically significant differences (p < 0.05)

When the failure type of the groups was determined using SEM (Figure 2), the unbleached group (Group 1) showed a predominantly cohesive and subsequent adhesive failure type. The group that underwent bonding immediately after bleaching was predominantly adhesive failures, followed by cohesive and mixed failure.

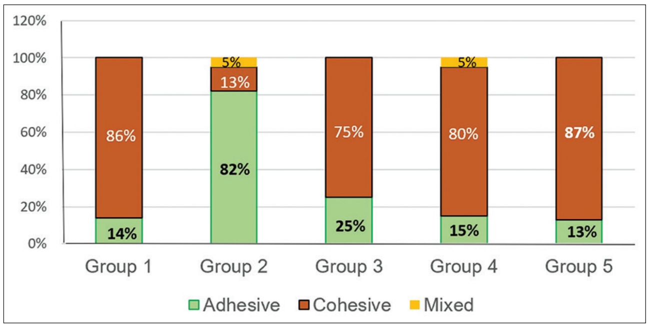

Figure 2: Distribution of failure modes (%) according to group

The SEM observation also showed that the 2-week waiting time after bleaching before restoration generated the majority cohesive, followed by adhesive types. Application using sodium ascorbate induced cohesive more than adhesive and mixed failures. Similar results occurred using chitosan nanoparticles, which exhibited a majority cohesive failure followed by adhesive. Figure 3 shows representative failure types of samples using SEM (at a magnification of ×300).

Discussion

This study used antioxidants before bonding procedures following external bleaching applied on enamel. These antioxidants could restrain the effect of free radicals released by the bleaching agent [11]. The results of this study were in accordance with the previous investigations that reported the application of a bleaching agent decreased SBS in all samples [10], [21]. It can be elucidated that reactive oxygen, which infiltrates into the tooth structures following bleaching, inhibits the infiltration of the resin and deteriorates its polymerization. As a result, bonding failures were noted in composite resin restorations performed immediately after applying bleaching agents [22]. Furthermore, the previous investigator also reported that SEM observation of the teeth restored following bleaching exhibited diminished resin tag establishment and an irregular arrangement of the hybrid layer due to bleaching [23]. The reduced bonding can also be due to prismatic arrangement damage, superficial surface demineralization, minerals disappearance, and organic matrix alteration. Changes in mineral composition could lower microhardness, which might induce lesser values of surface energy, resulting in reduced bond strength [24].

Antioxidants used before bonding procedures on bleached enamel might significantly counteract the opposing influences of bleaching agents and enhance the SBS [25]. The present study demonstrated that all antioxidants (sodium ascorbate and chitosan) could converse the unfavorable influences of bleaching agents on SBS. It is also verified that the effect of bleaching agents is temporary, and the enamel recovers its adhesiveness in the following 2 weeks. Following a 2-week waiting time, peroxide ions might decompose and replace hydroxyl radicals, then retire into the apatite lattice, leading to the removal of the structural changes produced by incorporating peroxide ions [8], [10]. The present findings are in conformity with previous studies showing that immersion of samples in artificial saliva or distilled water led to the recovery of decreased enamel bond strength. It was also considered that 2-week waiting time before applying the composite resin has the same effect as using antioxidants [3], [10].

Sodium ascorbate originates from a sodium salt of ascorbic acid and is a superior antioxidant. This antioxidant has the ability to lower free radicals [26]. The sodium ascorbate antioxidizing capability is supported to counteract and change the oxidizing impacts of the bleaching substance. As a result, the oxidized bonding substrate's changed redox ability is re-established, and the adhesive's polymerization persists without permanent termination [9], [11]. This study utilized 10% sodium ascorbate because of the efficient concentration employed as an antioxidant to counteract the free oxygen in the enamel's surface [27].

It has been known that chitosan has an antioxidant action. Previous research has indicated that chitosan hinders the ROS. As an antioxidant, chitosan has the ability to scavenge free radicals by donating hydrogen or single pairs of electrons. The chitosan scavenging process can be explained by the reaction of the action of hydrogen atoms, which react with superoxide and hydroxyl anion radicals, resulting in more stable radical macromolecules [28]. The composition of chitosan, specifically the hydroxyl (OH) and amino (NH_2) are the crucial elements for its antioxidant activity. Nevertheless, due to the semi-crystalline structure of chitosan, which has strong hydrogen interaction, it cannot be easy to separate hydroxyl and amino [29].

The molecular weight, concentration, and viscosity are directly proportional to the antioxidant

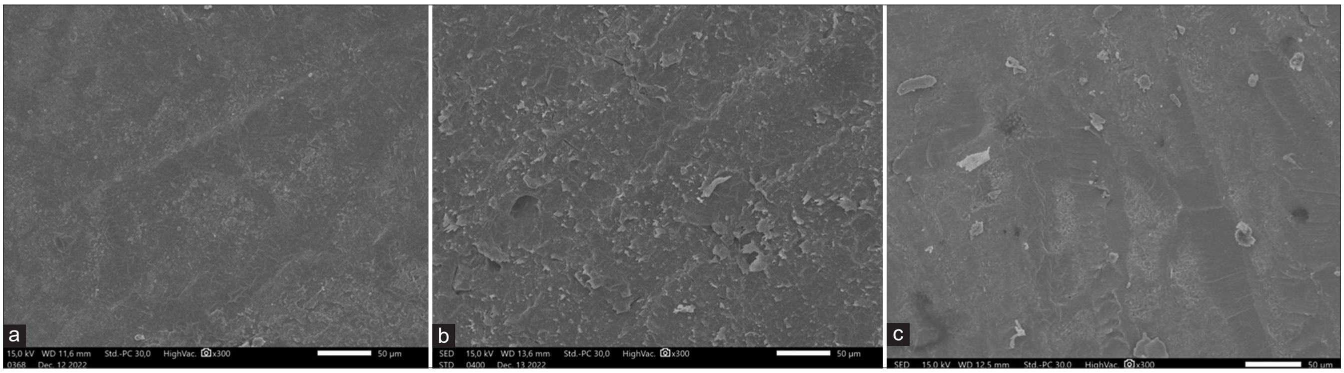

Figure 3: Representative scanning electron microscopy images of samples according to the failure types at ×300 magnification. (a) Adhesive type; (b) Cohesive type; (c) Mix of adhesive and cohesive

capability of chitosan. Chitosan is able to impede demineralization in enamel, avoids mineral loss, and keeps hydroxide apatite [30]. Chitosan enhances hydrophilicity, diminishes the fluid's contact angle with the enamel surface, and improves wettability, thus giving a more consistent dispersion to the enamel surface [15]. In addition, chitosan's greater SBS compared to sodium ascorbate can be explained by two mechanisms: Chitosan has antioxidant capability and remineralization action by preventing enamel demineralization [24]. The concentration of 0.2% chitosan used in this study exhibited a similar antioxidant effect to 10% sodium ascorbate.

The failure surface observed using SEM exhibited that most samples, which applied sodium ascorbate and chitosan nanoparticles, produced predominantly cohesive failure similar to the group with no bleaching and antioxidant application. This condition explains the high bond strength obtained after applying antioxidants, which was consistent with the fracture pattern. In contrast, the samples restored immediately using composite resin showed more adhesive than cohesive failure, which appears consistent with low SBS results. During a 2-week waiting time, samples showed cohesive failure occurred more frequently than adhesive failure, suggesting that enamel recovered its adhesiveness [17].

The present study has limitations. The first limitation is to control the direction of applied forces, which must be perpendicular to the sample's long axis, and the knife edge's direction must be loaded precisely at the edge between the composite resin restoration and enamel surface. The second limitation is the tooth structure used as a sample, which has individual characteristics. Further studies are needed to evaluate whether chitosan nanoparticles affect the whitening process, and the physical, chemical, and biological properties of enamel and dentine after the application of chitosan nanoparticles as an antioxidant to bleached teeth.

Conclusions

The chitosan nanoparticle has the potential as an antioxidant material since it produces similar SBS to composite resin restorations on enamel after external bleaching than the other groups, except for the bleached group that was directly restored using composite resin after the external bleaching treatment. Thus, chitosan nanoparticle is a promising antioxidant material in the future.

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