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RESEARCH ARTICLE

Effect of 35% sodium ascorbate on calcium and phosphorus loss in dentin bleached by 35% hydrogen peroxide

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ABSTRACT

Post bleaching procedures often have free radicals trapped in dentin tubule and interprismatic enamel, leading to demineralization and denaturation. Sodium ascorbate is an antioxidant substance known to bind residual free radicals to stop demineralization and denaturation. The purpose of the study was to assess the calcium and phosphorus loss from the dentin surface following bleaching by 35% hydrogen peroxide and reversal by 35% sodium ascorbate and the surface structure of the dentin. Six sound premolars were divided into their crown and root sections, with the crown subsequently being cut into four equal parts to obtain 24 samples. The calcium and phosphorus contents as well as the surface structure of the dentin were assessed using SEM-EDX. These were then divided into four groups, each containing six samples. Group A (control): the samples were bleached by 35% hydrogen peroxide, immersed in an artificial saliva, stored in an incubator at 37 °C for seven days. Group B: the samples were bleached by 35% hydrogen peroxide followed by the application of 35% sodium ascorbate for 5 minutes (once). Group C: the samples were bleached by 35% hydrogen peroxide followed by the applications of 35% sodium ascorbate (twice). Group D: the samples were bleached by 35% hydrogen peroxide followed by the applications of 35% sodium ascorbate (three times). The calcium and phosphorus contents as well as the surface structure of the dentin were re-assessed using SEM-EDX at the same locations. The results of one-way ANOVA indicated a difference in the calcium loss in the four treatment groups ($p<0.05$), but there was no difference in the phosphorus loss. An LSD test showed that there was a difference in the calcium loss between group A and groups C and D or between group B and groups C and D. The dentin tubules in group A were larger than group B, C and D. The frequency of 35% sodium ascorbate application had an effect on the calcium loss and the surface structure in the dentin bleached by 35% hydrogen peroxide.

Keywords: 35% sodium ascorbate; bleached dentin; 35% hydrogen peroxide; calcium and phosphorus loss

INTRODUCTION

A bleaching process is based on oxidation-reduction reactions. Bleaching material H_2O_2 produces unstable free radicals in the presence of radical hydroxyl (HO•), perhydroxyl radicals (HOO•), On (O•) radicals, perhydroxyl anion (HOO^-) and superoxide anion (O_2^-). The free radicals attack the color molecule by breaking the double bonds to produce simpler molecules.[1]

The bleaching procedure often contains ionic residues of perhydroxyl and O• in the dentin tubule which remains active for a certain period. Free radicals are highly electrophilic and unstable. A continuous bleaching process reaches a saturation point and at this point, a dental structural damage begins to occur because free radicals react with the organic and inorganic materials of enamel and dentin.[2]

Several studies have shown email changes due to bleaching, such as porosity and increased enamel depth.[3,4,5,6] Bleaching also decreases the elastic modulus of enamel, as reported by Azer et al.[7] Intracoronal bleaching causes side effects such as cervical resorption. Heithersay reported that 24.1% of cases of cervical resorption were caused by orthodontic treatment, 15.1% by dental trauma, 5.1% by surgery (transplantation or periodontal surgery) and 3.9% by intracoronal bleaching.[8] The combination between the intracoronal bleaching cause with one of the other causes results in 13.6% cervical resorption. A thermocatalytic technique can cause cervical resorption, with an incidence of 18-25%, while a

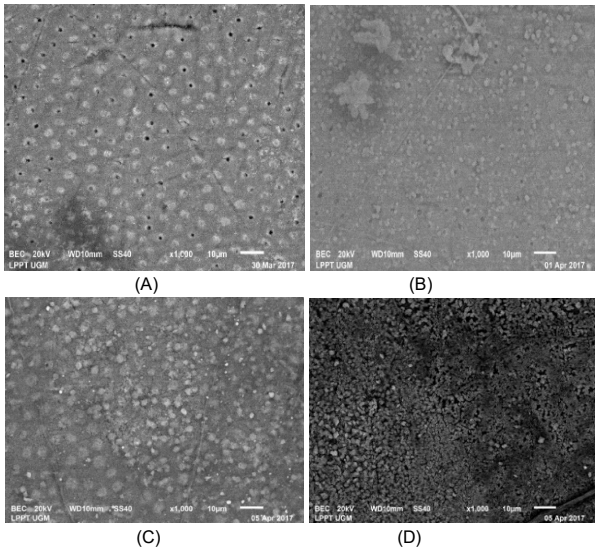

Figure 1. (A) Dentin structure post bleaching, soaked in saliva 1; (B) Dentin structure post bleaching, application of 35% SA 1x, (C) Dentin structure post bleaching, application of 35% SA 2x, (D) Dentin structure post bleaching, application of 35% SA 3x

walking bleach technique is relatively safer, with a 0-6% incidence.[9]

One attempt to accelerate free radical scavenging is the application of sodium ascorbate.[10] In intracoronal bleaching, the most commonly used material is 35% hydrogen peroxide. The amount of sodium ascorbate required to reduce hydrogen peroxide depends on the concentration of hydrogen peroxide used.[11,12] Freire and Murad also showed that an application with a longer duration does not affect the effectiveness of the reaction.[13,14] Application repetition is more effective than extending application time.[13] The study aimed to test the effect of frequency of 35% sodium ascorbate application on the mineral contents and structure of dentin bleached by 35% hydrogen peroxide.

MATERIALS AND METHODS

The research was done at LPPT unit IV UGM. Six premolar crowns were cut into four equal parts to obtain 24 samples. The samples were embedded in the gypsum stone, with the occlusal surface facing up, smoothed with sandpaper number 320 and 1000. The dentin surfaces, on which the calcium and phosphorus contents as well as surface structures were observed, were marked by 2B pencil. The samples were observed with SEM-EDX (SEM, JSM-T300, JEOL, Tokyo Jepang), with magnification 100.1000 and 5000 times. Observations were made on 5 areas around the marked surfaces.

The samples were applied by 0.025 ml of 35% hydrogen peroxide (Opalescene Endo, Ultradent Products, South Jordan,UT, USA), stored in an incubator at 37 °C for 120 hours in closed tubes, where one tube was used for one sample, then washed and dried. Subsequently the 24 samples were divided into 4 groups: group A was not applied by sodium ascorbate, immersed in artificial saliva and stored in an incubator at 37 °C for 7 days; group B was applied by 0.025 ml of 35% sodium ascorbate (Sigma Eldrich, Singapura) 5 min, washed and dried; group C was applied by 35% sodium ascorbate and the same procedure was repeated twice; group D was applied by 35% sodium ascorbate and the same procedure was repeated three times. Furthermore the calcium and phosphorus contents as well as the surface

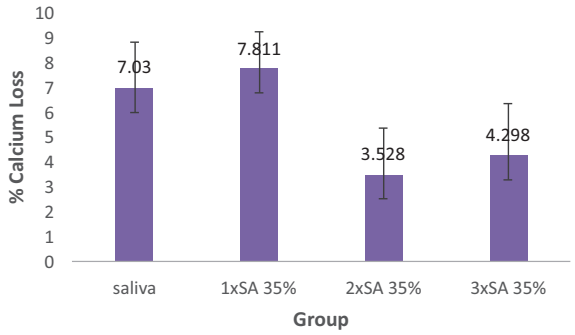

Figure 2. The mean calcium loss

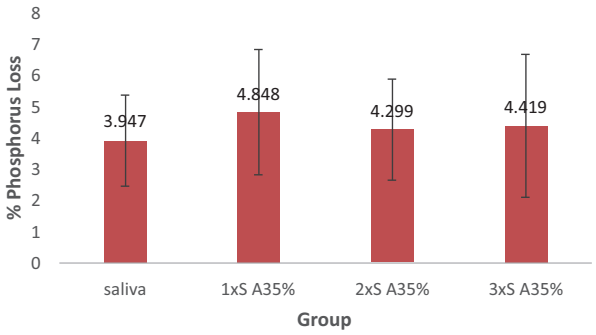

Figure 3. The mean phosphorus loss

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Table 1. ANOVA test of the calcium loss from the dentin surface following bleaching by 35% hydrogen peroxide and reversal by 35% sodium ascorbate

Variables	Sum of square	SD	Mean square	F	p
Among group	77.424	3	25.808	7.738	0.001
Inner group	66.707	20	3.335		
Total	144.131	23			

Table 2. LSD test of the calcium loss from the dentin surface following bleaching by 35% hydrogen peroxide and reversal by 35% sodium ascorbate

Group	LSD
Saliva (A) - SA 35% 1X (B)	-0.78167
Saliva (A) - SA 35% 2X (C)	3.50133*
Saliva (A) - SA 35% 3 X (D)	2.73200*
SA 35% 1X (B) - SA 35% 2X (C)	4.28300*
SA 35% 1X (B) - SA 35% 3X (D)	3.51367*
SA 35% 2X (C) - SA 35% 3X (D)	-0.76933

Table 3. ANOVA test of the phosphorus loss from the dentin surface following bleaching by 35% hydrogen peroxide and reversal by 35% sodium ascorbate

Variables	Sum of square	SD	Mean square	F	p
Among group	2.485	3	0.828	0.232	0.873
Inner group	71.345	20	3.567		
Total	73.870	23			

structure (dentin tubule diameter) of each group were re-observed using EDX-SEM, at the same location prior to the treatment. The data were statistically analyzed with One-Way ANOVA and LSD.

RESULTS

The structure as well as the calcium and phosphorus losses from the dentin surface following bleaching by 35% hydrogen peroxide and reversal by 35% sodium ascorbate were observed with SEM-EDX. The structures of the dentin are shown in Figure 1.

The mean calcium and phosphorus losses are shown in Figures 2 and 3. The normality and homogeneity tests showed that the data were distributed normally and homogenous. Table 1 shows the mean, standard deviation, and significance from the one-way ANOVA of the calcium loss.

Table 2 shows significant differences ($p<0.05$) in the calcium loss from post hoc LSD among the 4 experimental groups. Table 3 shows the mean, standard deviation, and significance from one-way ANOVA of the phosphorus loss.

DISCUSSION

The research showed a decrease in the percentage of calcium and phosphorus in all the groups. The lowest decrease in the calcium percentage was found in the group applied by 35% sodium ascorbate twice (3.528%). The highest calcium decrease was found in the group applied by 35% sodium ascorbate once (7.81%). In addition, the highest decrease in the phosphorus percentage was found in

the group applied by 35% sodium ascorbate once (4.848%). The lowest decrease in the phosphorus percentage was in the group soaked in saliva for 1 week (3.947%). The decrease in the calcium percentage was higher than that in the phosphorus percentage. This result is in accordance with Maleknejad et al.[9] and Cakir et al.[15] Cakir et al.,[15] demonstrating that 35% hydrogen peroxide application on dentine for 15 minutes caused a reduction in calcium by 5.1% and a decrease in phosphorus by 2%. In this research, 35% hydrogen peroxide was applied much longer (2x120 hours) with a higher volume because the application was repeated twice. The dentin exposed to hydrogen peroxide in a longer time and higher volume is more likely to experience a higher decrease in calcium and phosphorus.

The ANOVA test showed that there was a significant difference of the calcium percentage in the groups applied by sodium ascorbate 35% 1, 2, 3 times, and the group soaked in saliva for 1 week. The decrease in the calcium and phosphorus percentage in bleached dentin is due to demineralization. Free radicals resulting from the breakdown of hydrogen peroxide are very electrophilic and unstable because they do not have any electron pairs, so the free radicals form electron pairs to be stable. In their effort to form the electron pairs to be stable, the free radicals react with chromogen as well as dentin organic and inorganic molecules. In addition, the free radicals also react with the double bonds of chromogenic molecules, causing the molecules to break down into simpler molecules that are less reflective, causing the color of teeth to be lighter.[1,16,17] The free radicals present in the bleached dentin still seek electron pairs so they react with the organic and inorganic molecules of the dentin. When the free radicals react with the inorganic molecules of the dentin, demineralization occurs. The reaction of hydrogen peroxide with hydroxyapatite which is the main inorganic material of the dentine can be described as follows:

$$H_2O_2 + Ca_{10}(PO_4)_6(OH) \rightarrow 10CaO + 3P_2O5 + H_2O.$$[18]

The different calcium and phosphorus percentages in the four treatment groups were probably due to the different amounts of residual free radicals resulting from the breakdown of 35% hydrogen peroxide in the four treatment groups. The different amounts of the residual free radicals will cause different demineralization levels.

Post hoc LSD test showed that the decrease in the calcium percentage of the group soaked in saliva for 1 week was higher than those of the groups applied with 35% sodium ascorbate twice and three times. The decrease in the calcium percentage of the group applied with 35% sodium ascorbate once was higher than those of the groups applied with 35% sodium ascorbate twice and 3 times. The results of this study are in line with the research reported by Freire et al. Freire et al., demonstrating that to remove all the residual free radicals, 35% sodium ascorbate should be applied more than once. If all the residual free radicals can be removed, then the post-bleaching demineralization process stops.[13]

This study showed that applying 35% sodium ascorbate twice on dentin bleached by 35% hydrogen peroxide can stop demineralization, proven by the fact that the decrease in the calcium content of this group was lower and significantly different from the group without the application of 35% sodium ascorbate and the groups with 35% sodium ascorbate applied once. However, when 35% sodium ascorbate was applied 3 times, the decrease in the calcium content was not significantly different from the group where 35% sodium ascorbate was applied twice. Referring to these results, it is assumed that the residual free radicals after bleaching with 35% hydrogen peroxide were all bond when 35% sodium ascorbate was applied to the dentin twice.

This study showed that there was no significant difference in the decrease in the calcium and phosphorus percentage between the group applied with 35% sodium ascorbate once and the group soaked in saliva. This is probably due to the fact that the bleached dentins in these two groups

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contained the same amount of free radicals. Applying 35% sodium ascorbate only once was not able to bind all the residual free radicals of 35% hydrogen peroxide. One week saliva immersion allowed for the release of residual free radicals in the dentin tubules, as demonstrated by Sunfield et al.,[19] Camps et al.[20] and Freire et al.[13] According to Sunfield et al.,[19] immersion time in saliva affects the removal of residual peroxide. Camps et al.[20] showed that high free radicals are released 1 hour post bleaching and the release lasts 24 hours post bleaching, then decreases after 48 hours and reaches the minimum after 120 hours. Freire et al.[13] also stated that the length of time a specimen immersed in water affects the amount of residual free radicals in dentine. This study further demonstrated that large amounts of free radicals were released post bleaching and increased for the first 24 hours then gradually decreased after 48 hours. Reduced residual hydrogen peroxide during a 7 day delay can also be attributed to the unstable properties of hydrogen peroxide[21] that can decompose into water and oxygen.[22]

Another possibility in the dentin group soaked in saliva is demineralization, followed with remineralization because saliva contains calcium and phosphorus. The results of this study indicated that applying 35% sodium ascorbate for one time prevents further demineralization, similar to immersion in saliva for 1 week.

The dentin bleached by 35% hydrogen peroxide and soaked in artificial saliva for 1 week caused the dentin tubules to have a larger diameter than before bleaching. This condition occurred because soaked in saliva for 1 week made the remaining free radical release. There was a very high amount of residual free radicals shortly after bleaching which then gradually decreased. Residual free radicals inside the dentin tubules attack the inorganic or organic molecules containing double bonds. The organic material of dentin consists of collagen and non collagen proteins; collagen proteins consist mainly of type I collagen and a small fraction of V-type collagen. Non collagen protein consists of dentin phosphoprotein (DPP), dentin protein matrix 1 (DMP1), dentin sialoprotein (DSP), osteoprotein (OPN), osteocalcin and Bone sialoprotein (BSP). If residual free radicals attack the proteins, denaturation occurs. Denaturation causes a damage or loss of collagen in the intratubular and intertubular dentin. The dentinal tubules are dyed by intratubular dentin. Intratubular dentin matrix contains fewer collagen fibers and more sulfated proteoglycans and minerals than intertubuler dentin.[21] Intratubular dentin is more soluble than intertubular dentin because it contains less collagen. Dissolving or damaging the intratubular dentin causes the dentinal tubules to have a larger diameter.

The dentin bleached by 35% hydrogen peroxide and applied by 35% sodium ascorbate for one time showed dentin tubules with a larger diameter although not as large as those of the group soaked in saliva for 1 week. This condition might be due to the fact that applying 35% sodium ascorbate for one time still left enough residual free radicals to attack the inorganic and organic molecules of the dentin. The intratubular collagen denaturation in the dentin applied by 35% sodium ascorbate for one time was still high enough and the dentinal tubules were open. The condition was different from the dentin applied with 35% sodium ascorbate for two or three times. In these groups, the dentinal tubules were not wide open, covered by a layer which was most likely to be collagen. The few residual free radicals in these groups were likely to cause only a slight demineralization of the inorganic material and collagen denaturation in the intra-tubular dentin. However, this research only used a limited number of samples, so it is suggested to use more samples in future research.

CONCLUSIONS

Based on the research that has been done, the conclusions of this research are: 1). The application of 35% sodium ascorbate to the dentin bleached by 35% hydrogen peroxide caused a lower decrease in the calcium content and a smaller dentin tubulus diameter; 2). The decrease in the calcium content

in the dentin bleached by 35% hydrogen peroxide and applied by 35% sodium ascorbate for two and three times was lower than that in the group applied with 35% sodium ascorbate for only one time; 3). The dentin tubulus diameter of the dentin bleached by 35% hydrogen peroxide and applied by 35% sodium ascorbate for two or three times was smaller than that of the dentin applied with 35% sodium ascorbate for one time.

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