Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2015 March; 48(1): 7–11

Research Report

Effect of casein phosphopeptide-amorphous calcium phosphate on the flexural strength of enamel-dentin complex following extracoronal bleaching

Diatri Nari Ratih and **Hendargo Agung Pribadi**
Department of Conservative Dentistry
Faculty of Dentistry, Universitas Gadjah Mada
Yogyakarta - Indonesia

ABSTRACT

Background: *Bleaching can affect the mechanical properties of enamel-dentin complex, such as flexural strength. Casein phosphopeptide-amorphus calcium phosphate (CPP-ACP) is often used following bleaching treatment to reduce hypersensitivity and to increase demineralization of tooth.* ***Purpose:*** *The purpose of the study was to investigate the effect of CPP-ACP on the flexural strength of enamel-dentin complex following extracoronal bleaching.* ***Methods:*** *Forty-eight enamel-dentin plates (size 8 x 2 x 3 mm) were randomly assigned into 6 groups, each consisted of 8 samples. Group 1, no bleaching and immersed in artificial saliva. Group 2, no bleaching, CPP-ACP application only. Group 3, bleaching using 15% carbamide peroxide. Group 4, similar to group 3, except application of CPP-ACP for the times between bleaching. Group 5, bleaching with 40% hydrogen peroxide. Group 6, similar to group 5, except application of CPP-ACP for the times between bleaching. Flexural strength of each enamel-dentin plate was tested by three-point bending test using universal testing machine.* ***Results:*** *The results showed that 15% carbamide peroxide and 40% hydrogen peroxide significantly reduced flexural strength of enamel-dentin (216.25±26.44 MPa and 206.67±32.07 MPa respectively). Conversely, application of CPP-ACP following both bleachings increased flexural strength (266.75± 28.27MPa and 254.58±36.59 MPa respectively). A two-way Anova revealed that extracoronal bleaching agents significantly reduced flexural strength ($p<0.05$), while application of CPP-ACP significantly increased flexural strength of bleached enamel-dentin complex ($p<0.05$).* ***Conclusion:*** *Extracoronal bleaching agents reduce flexural strength, whereas application of CPP-ACP following bleaching either with 15% carbamide peroxide or 40% hydrogen peroxide can increase the flexural strength of enamel-dentin complex.*

Keywords: *Extracoronal bleaching; hydrogen peroxide; carbamide peroxide; CPP-ACP; flexural strength*

Correspondence: Diatri Nari Ratih, c/o: Departemen Konservasi Gigi, Fakultas Kedokteran Gigi Universitas Gadjah Mada. Jl. Denta 1, Sekip Utara Yogyakarta 55281, Indonesia. E-mail: trinaugm@yahoo.com

INTRODUCTION

Tooth discoloration, especially in anterior teeth, often cause aesthetic problems. This color change can affect a patient's self confidence; therefore patient seeks treatment to improve the color of teeth. With careful diagnosis, appropriate treatment planning and attention to technique, bleaching can be considered a conservative and safe procedure to lighten discolored teeth.[1]

Bleaching agents, including carbamide and hydrogen peroxide, act as a poweful oxidizing agents and can give rise to agents known to be effective bleaching agents (*i.e.*, its corresponding mono-anion ($H0_2^-$) and hydroxyl radical (OH)). Moreover, carbamide peroxide also releases urea, which is rapidly decomposed into carbon dioxide and ammonia. Chemical reaction of the two reagents with the organic extracellular matrix components, including pigments or chromophores, constitutes the chemical basis of tooth bleaching.[2,3]

Although bleaching procedures are intended to be applied topically to the enamel surface, the effects of such procedures are not necessarily restricted to enamel only, but also to dentin and pulp.[3] Bleaching methods generally include the application of 10% to 15% carbamide peroxide or 30% to 38% hydrogen peroxide.[2,4] Recently, 40% hydrogen peroxide has been introduced to the market 40 a bleaching agent and become more populer than previous lower consentration of bleaching agents.[4]

A general concern in bleaching procedures relates to the possible weakening of tooth structure subsequent to bleaching procedures. It has been reported that changes in enamel surfaces charactheristic such as increased porosity,[5] erosion,[6] increased roughness,[7] decreased hardness,[8] demineralization of tooth structures,[9] depression and superficial irregularities,[10] after bleaching procedures. Furthermore, alterations in the mechanical properties, including tensile strength, flexural strength, fracture toughness of dental tissues particularly in enamel and dentin following to bleaching have been reported in several studies.[11-13]

Extracoronal bleaching can be associated with several complications, including tooth sensitivities, which has been reported in 15% to 78% of patients undergoing such procedures.[10,11] Various treatments have been undertaken to manage tooth sensitivity, including the use of oxalates and nitrates of potassium or a combination of potassium nitrate and fluoride, as well as the use of amorphous calcium phosphates.[11]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is ACP complexed with CPP, which is from the major protein of milk. CPP-ACP and localizes ACP in dental plaque, thereby concentrating calcium and phosphate on the tooth surface.[14-16] By maintaining a high-concentration gradient of calcium and phosphate ions, CPP-ACP assists to suppress demineralization and promotes remineralization of enamel by the deposition of apatite.[17-19] Previous studies demonstrated that CPP-ACP has been able to remineralize human enamel subsurface lesions. The remineralized enamel has also been shown to be more resistant to subsequent acid attack.[16,17,20,21]

Recently, CPP-ACP has been used as a paste, in conjuction with or following treatments such as bleaching in order to decrease tooth sensitivity. The effect of applying CPP-ACP to enamel surfaces on bond strength subsequent to bleaching procedures has been investigated in numerous studies.[22-24] A previous study also showed a significant reduction in bovine dentin flexural strength and modulus of elasticity after a 2-week direct application of 10% and 15% carbamide peroxide.[9] Application of CPP-ACP following bleaching treatment (in the absence of saliva) could compesate for decreased flexural strength of the bovine enamel-dentin complex.[12]

Although several studies have evaluated the effects of CPP-ACP, no study has been undertaken to evaluate the flexural strength following application of extracoronal bleaching agents on human enamel-dentin complex. Therefore, the purpose of this study was to investigate the effect of CPP-ACP on the flexural strength of enamel-dentin complex following extracoronal bleaching.

MATERIALS AND METHODS

Forty-eight human premolars, extracted within the previous 3 months, immediately stored in distilled water at 4^0 C until the study was conducted. Rectangular blocks measuring 8 mm in length, 2 mm in width, and 3 mm in height were prepared from the middle part of facial surfaces of the extracted premolars using a cutting machine (Microtome, Leica, Wetzlar, Germany). The specimens were randomly assigned into 6 groups, each consisted of 8 samples. Group 1, comprised the control group, which received no bleaching treatment and immersed in artificial saliva. Group 2, specimens were not received bleaching treatment, but were applied with CPP-ACP (Tooth Mousse, GC Inc, Tokyo, Japan) for 30 minutes, twice daily for 14 days. Group 3, specimens were bleached using 15% carbamide peroxide (15% Opalescence PF, Ultradent, South Jourdan, UT., USA) for 30 minutes, twice daily for 2 consecutive weeks. Group 4, similar to group 3, except specimens were applied CPP-ACP for the times between bleaching (30 minutes twice daily for 2 consecutive weeks). Group 5, specimens were bleached using 40% hydrogen peroxide (40% Opalescence Boost, Ultradent,

Figure 1. Three-point bending test using Universal testing machine.

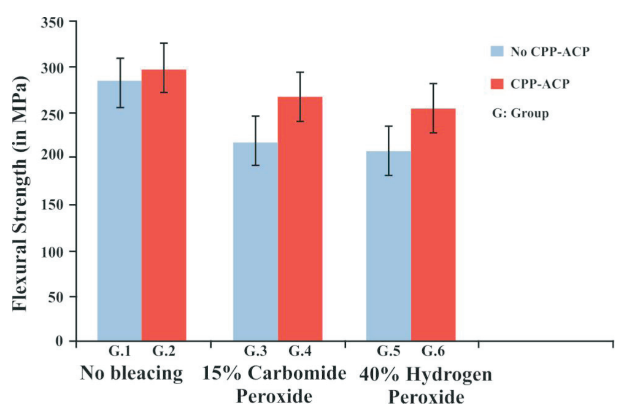

Figure 2. The mean and standard deviation of enamel-dentin flexural strength after applicationof bleaching agents and CPP-ACP (in MPa).

South Jourdan, UT, USA) for 1hour/day, twice weekly for 2 consecutive weeks. After each bleaching session, the specimens were thoroughly rinsed with distilled water for 30 seconds and stored in artificial saliva. Group 6, similar to group 5, except specimens were applied CPP-ACP for the times between bleaching (30 minutes, twice daily for 2 consecutive weeks).

All specimens were then rinsed using distilled water and incubated in artificial saliva for 24 hours at 37^0 C before being tested. The specimens were subjected to a three-point bending test on a Universal testing machine (type AMU-5-DE, Tokyo Testing Machine, MFG, Co., LT, Tokyo, Japan) that applied force to the center of the enamel side of the specimens at a crosshead speed of 1 mm/min (Figure 1). The mounting apparatus consisting of two rods, mounted parallel, with 6 mm between the centers. The maximum load supported by the specimen prior to failure was used to calculate the flexural strength value. Flexural strength was determined using the following formula:[10] Flexural strength (in MPa)= $3P_fL/2WH^2$, where P_f is the measured maximum load at the time of specimen fracture, L is the distance between supports on the tension surface (6 mm), W is the mean specimen width, H is the mean height of the specimen between the tension and compression surfaces. Results were subjected to statistical analysis using a two way Anova and a post-hoc Tukey test with 95% level of significance.

RESULTS

The mean and standard deviation of flexural strength values (in MPa) are depicted in Figure 2. The results showed that flexural strength decreased in enamel-dentin complex, which was bleached either using 15% carbamide peroxide or 40% hydrogen peroxide (group 3 and group 5) as compared to control group, which was soaked in artificial saliva only. On the other hand, application of CPP-ACP on to bleached specimens resulted in the increase of flexural strength (group 4 and group 6). Specimens that were applied CPP-ACP without bleaching treatment revealed the highest flexural strength (297.17±28.29 MPa) compared to the other groups. Conversely, specimens which were bleached with 40% hydrogen peroxide and without application of CPP-ACP showed the lowest flexural strength (206.67 ±32.07 MPa).

A two way anova demonstrated that bleaching using either 15% carbamide peroxide or 40% hydrogen peroxide, as well as CPP-ACP application influenced on the flexural strength of enamel-dentin complex ($p<0.05$). On the other hand, no interaction occured between bleaching and application of CPP-ACP ($p>0.05$). Multiple comparisons by a post-hocTukey test revealed that all groups showed significant differences ($p<0.05$), except between group 1and group 4, group 2 and group 4, group 4 and group 6 ($p>0.05$).

DISCUSSION

The results of this study indicated that reduction in the flexural strength values of the enamel-dentine complex occured after the extracoronal bleaching with either 15% carbamide peroxide or 40% hydrogen peroxide. The reduction in flexural strength was probable attributed to changes caused by bleaching agents in the inorganic or organic component of dentin. Enamel contains 98% inorganic and 2% organic materials, while dentin consists of 70% inorganic, 20% organic materials and 10% water,[24] therefore application of bleaching agents on to enamel-dentin complex may cause demineralization of inorganic component of enamel-dentin complex and resulting in decreasing flexural strength. In addition, the redox reaction of bleaching agents can lead to dissolution of organic and inorganic materials teeth structure and the remaining is only carbon dioxide and water.[22] In other words, bleaching agent is capable to alter the ratio of Ca/P of tooth structure,[9] which in turns, reducing flexural strength.

Additionally, urea and peroxide have ben associated with proteolytic reaction, which is due to induce denaturating dentin collagen and oxidizing dentin protein, resulting in degradation of proteins.[2] Urea can penetrate not only the enamel surface but also interprismatic of enamel. The penetration increases the permeability of the enamel and subsequently alters the microstructural of enamel.[13] Bleaching agents improved access to intratubular minerals by dispersing collagen fibrils. Loss of minerals leads to loss of the binding matrix. Therefore, the destruction of organic and mineral parts of dentin occurs continuously, resulting in decreasing flexural strength.[12] Moreover, some of the structural alterations in bleached dentin have been attributed to alterations in the water, mineral, and collagen content of dentin and non-collagenous protein.[3]

In clinical situations, bleaching agents are usually applied to enamel rather than directly applied to dentin.[21,22] However, peroxide ions are able to penetrate through enamel and dentin through a capillary rise in enamel interprismatic spaces, convective mass transfer, or classic molecular diffusion based on a molecular path, and form measurable amounts of bleach within the tooth pulp.[14] Thus, enamel-dentin plates were used in this study as specimens to mimic clinical condition.

In this study, the application time of 15% carbamide peroxide and 40% hydrogen peroxide were 30 minutes and 1 hour respectively, since dentin in that period may be affected by bleaching agent. This result is in accordance with Tam *et al.*[3] who reported that bleaching agent diffused through enamel within 15 to 25 minutes. Bleaching agents influence the enamel by affecting enamel organic matrix with the action of free radicals.[14] The porosities created by the bleaching agent along the exposed area of enamel may act as stress raisers during a three-point bending tests, resulting in an early fracture during fracture resistance investigations.[3] Enamel could reduce the effects of hydrogen peroxide on dentin.[25] Several factors such as the enamel thickness, the existance of enamel fractures and cracks, dentin permeability and the direction of dentinal tubules affected the diffusion of bleaching agents through dentin.[18]

This present study showed a significant difference in flexural strength occured between 15% carbamide peroxide and 40% hydrogen peroxide ($p<0.05$). Nevertheless the influence of 40% hydrogen peroxide bleaching was slightly greater than that of 15% carbamide peroxide on the flexural strength of enamel-dentin complex. This difference is likely due to pH of both bleaching agents. It has been reported that 40% hydrogen peroxide having a pH that is slightly lower than the 15% carbamide peroxide. Acidic pH can increase the demineralization of inorganic materials in enamel-dentin complex,[23] which in turns, reduce the flexural strength of enamel-dentin complex.[13]

The results of this study also revealed that application of CPP-ACP increased flexural strength of enamel-dentin complex, which was bleached with 15% carbamide peroxide and 40% hydrogen peroxide, although it was less than normal dentin. This material acts as a source of calcium and phosphate for enamel since CPP-ACP contains calcium 13 mg/g, phosphate 5.6 mg/g, with a pH of 7.8.[16,17] CPP-ACP could increase enamel mineralization and prevent demineralization by buffer mechanism.[15] Casein is a buffer for plaque acid, and it leached amino acids that can receive proton ions. The presence of the CPP-ACP on the surface of the enamel-dentin complex is able to act as a barrier that prevent protons to diffuse.[4,19] In addition, casein phosphopeptide is capable to stabilize the calcium-phosphate on the tooth surface, thereby maintaining the calcium-phosphate concentration remains high on the tooth surface.[15] In clinical situation, application of the CPP-ACP on enamel surface can also lead this material to bind with biofilms, plaque, bacteria, hydroxyapatite and surrounding soft tissues, hence it might be always provide calcium and phosphate for teeth.[18] The duration of CPP-ACP application and the existence of several factors such as saliva may also affect the results of the present study.[4]

This study used artificial saliva to simulate the clinical condition. Since CPP-ACP can also stimulate the flow of saliva, it might improve the effectiveness of CPP-ACP in clinical conditions. Immersion in artificial saliva between the applications of bleaching agents can prevent demineralization of the enamel and dentin rather than only soaked in distilled water between the times the application of bleaching agents.[10] It is because saliva has buffering properties and prevents demineralization, it is also rich in calcium and phosphate and acts as a source of remineralization after bleaching procedures.[15] On the contrary, the distilled water does not contain calcium and phosphate as in artificial saliva. It seems that the effect of CPP-ACP might be greater in clinical conditions than in vitro study.[21]

CPP-ACP can also reduce the hypersensitivity following bleaching treatment.[17] This phenomenon is due to increase mineral crystals deposition in the enamel, hence patients who treated bleaching did not experience of dentin hypersensitivity. Additionally, the application of CPP-ACP did not affect the effectiveness of bleaching procedures.[19]

Based on the results of this study, it can be concluded that extracoronal bleaching agents reduce flexural strength, whereas CPP-ACP following bleaching either with 15% carbamide peroxide or 40% hydrogen peroxide can increase the flexural strength of enamel-dentin complex.

ACKNOWLEDGMENT

This study was funded by Universitas Gadjah Mada Faculty of Dentistry Research Grant.

REFERENCES

1. Polydorou O, Hellwig E, Auschill TM. The effect of different bleaching agents on the surface texture of restorative materials. J Op Dent 2006; 31(4): 473-80.
2. Goldberg M, Grootveld M, Lynch E. Undesireable and adverse effects of tooth-whitening products: A review. Clin Oral Invest 2010; 14(1): 1-10.
3. Tam LE, Kuo VY, Noroozi A. Effect of prolonged direct and indirect peroxide bleaching on fracture toughness of human dentin. J Esthet Restor Dent 2007; 19(2): 100-10.
4. Cunha AGG, Vasconcelos AAM, Borges BCD, Vitoriano JDO, Alves C, Machado CT, Santos AJS. Efficacy of in-office bleaching techniques combined with the application of a casein phosphopeptide-amorphous calcium phosphate paste at different moments and its influence on enamel surface properties. Micros Res Tech 2012; 1(1): 1-7.
5. Ferreira SS, Araujo JL, Morhy ON, Tapety CM, Youssef MN, Sobral MA. The effect of flouride therapies on the morphology of bleached human dental enamel. Micros Res Tech 2011; 74(5): 512-6.
6. Martin JM, Almeida JB, Rosa EAR, Soares P, Torno V, Rached RN, Mazur RF. Effect of fluoride therapies on the surface roughness of human enamel exposed to bleaching agents. Quintessence Int 2010; 41(1): 71-8.
7. Borges AB, Samezima LY, Fonseca LP, Yui KCK, Borges ALS, Toreres CRG. Influence of potentially remineralizing agents on bleached enamel microhardness. Oper Dent 2009; 34(5): 593-7.
8. Potocnik I, Kosec L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content. J Endod 2000; 26(2): 203-6.
9. Tam LE, Lim M, Khanna S. Effect of direct peroxide bleaching application to bovine dentin on flexural strength and modulus in vitro. J Dent 2005; 33(4): 451-8.
10. Tam LE, Abdool R, El-Badrawy W. Flexural strength and modulus properties of carbamide peroxide-treated bovine dentin. J Esthet Restor Dent 2005; 17(3): 359-68.
11. Tam LE, Noroozi A. Effects of direct and indirect bleach on dentin fracture toughness. J Dent Res 2007; 86(10): 1193-7.
12. Khoroushi M, Mazaheri H, Manoochehri AE. Effect of CPP-ACP application on flexural strength of bleached enamel and dentin complex. Oper Dent 2011; 36(4): 372-9.
13. Tredwin CJ, Naik S, Lewis NJ, Scully J. Hydrogen peroxide tooth-whitening (bleaching) products: Review of adverse effects and safety issues. British Dent J 2006; 200(4): 371-6.
14. Kowalcsyk A, Botulinski B, Jaworska M, Kierklo A, Pawinska M, Dabros E. Evaluation of the product based on recaldent technology in the treatment of dentin hypersensitivity. Adv Med Sci 2006; 51 (suppl): 40-2.

15. Ratjitkar S, Rodrigueez JM, Kaidonis JA, Richards LC, Townsend GC, Bartlett DW. The effect of casein phosphopeptide-amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion. J Dent 2009; 37(2): 250-4.
16. Reynolds EC, Cai F, Cohrane NJ, Shen F, Walkel GD, Morgan MV. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2008; 87(3): 344-8.
17. Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate: The scientific evidence. Adv Dent Res 2009; 21(1): 25-9.
18. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: A systemic review of the literature. JADA 2012; 139(7): 815-924.
19. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphates on remineralization of artificial caries-like lesion: An in vitro study. Aust Dent J 2008; 53(1): 34-40.
20. Moule CA, Angelis F. Resin bonding using an all-etch or self-etch adhesives to enamel after carbamide peroxide and/or CPP-ACP treatment. Aust Dent J 2007; 52(2): 133-7.
21. Adebayo OA, Burrow MF, Tyas MJ. Dentin bonding after CPP-ACP paste treatment with and without conditioning. J Dent 2008; 36(12): 1013-24.
22. Soldani P, Amaral CM, Rodrigues JA. Microhardness evaluation of in situ vital bleaching and thickening agents on human dental enamel. Int J Periodontics Restorative Dent 2010; 30(2): 203-11.
23. Tang B, Millar BJ. Effect of chewing gum on tooth sensitivity following whitening. British Dent J 2010; 208(5): 571-7.
24. Avery JK, Chiego DJ. Essentials of oral histology and embryology. 3^{th} ed. St. Louis: Mosby Elsevier; 2006. p. 55-103.
25. Date RF, Yue J, Barlow AP, Bellamy PG, Prendergast MJ, Gerlach RW. Delivery, substantivity and clinical response of a direct application percarbonate tooth whitening film. Am J Dent 2006; 16(1): 3B-8B.