

Research Report

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

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ABSTRACT

Background: Bleaching can affect the mechanical properties of enamel-dentin complex, such as flexural strength. Casein phosphopeptide-amorphous 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 ± 28.27 MPa 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.

Key words: Extracoronal bleaching, hydrogen peroxide, carbamide peroxide, CPP-ACP, flexural strength

ABSTRAK

Latar belakang: Perawatan bleaching dapat berpengaruh terhadap sifat mekanis gigi seperti kekuatan fleksural. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) sering digunakan pada gigi pasca bleaching untuk mengurangi hipersensitivitas dan remineralisasi email dan dentin. **Tujuan:** Tujuan dari penelitian ini adalah untuk mengetahui pengaruh CPP-ACP terhadap kekuatan fleksural kompleks email-dentin pasca bleaching ekstrakoronal. **Metode:** Empat puluh delapan pelat email-dentin (ukuran 8 x 2 x 3 mm) dibagi secara random menjadi 6 kelompok, masing-masing terdiri dari 8 gigi. Kelompok 1, tidak dilakukan bleaching dan direndam dalam saliva tiruan. Kelompok 2, tidak dilakukan bleaching, diaplikasikan CPP-ACP. Kelompok 3, dilakukan bleaching dengan karbamid peroksida 15%. Kelompok 4, dilakukan bleaching seperti kelompok 3, kecuali diantara waktu bleaching diaplikasikan CPP-ACP. Kelompok 5, dilakukan bleaching dengan hidrogen peroksid 40%. Kelompok 6, dilakukan bleaching seperti kelompok 5, kecuali diantara waktu bleaching diaplikasikan CPP-ACP. Setiap pelat email-dentin dilakukan uji three-point bending menggunakan universal testing machine untuk mengetahui kekuatan fleksural. **Hasil:** Bahan bleaching karbamid peroksida 15% dan hidrogen peroksid 40% menurunkan kekuatan fleksural email-dentin kompleks (masing-masing $216,25 \pm 26,44$ MPa dan $206,67 \pm 32,07$ MPa). Namun aplikasi CPP-ACP pasca bleaching dapat meningkatkan kekuatan fleksuralnya ($266,75 \pm 28,27$ MPa dan $254,58 \pm 36,59$ MPa). ANOVA dua jalur menunjukkan bahwa bleaching ekstrakoronal menurunkan kekuatan fleksural secara signifikan ($p < 0,05$).

sedangkan aplikasi CPP-ACP pada kompleks email-dentin yang telah dilakukan bleaching dapat meningkatkan kekuatan fleksural secara signifikan ($p < 0,05$). **Simpulan:** Bahan bleaching dapat menurunkan kekuatan fleksural kompleks email-dentin, sedangkan aplikasi CPP-ACP dapat meningkatkan kekuatan fleksural pada kompleks email-dentin yang telah dilakukan bleaching.

Kata kunci: Bleaching ekstra koronal, hidrogen peroksida, karbamid peroksida, CPP-ACP, kekuatan fleksural

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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 powerful oxidizing agents and can give rise to agents known to be effective bleaching agents (*i.e.*, its corresponding mono-anion (HO_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 popular than previous lower concentration 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 characteristic 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

Extracoronary 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 conjunction 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 compensate 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 extracoronary 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 extracoronary bleaching.

MATERIALS AND METHODS

Forty-eight human premolars, extracted within the previous 3 months, immediately stored in distilled water at 4°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



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

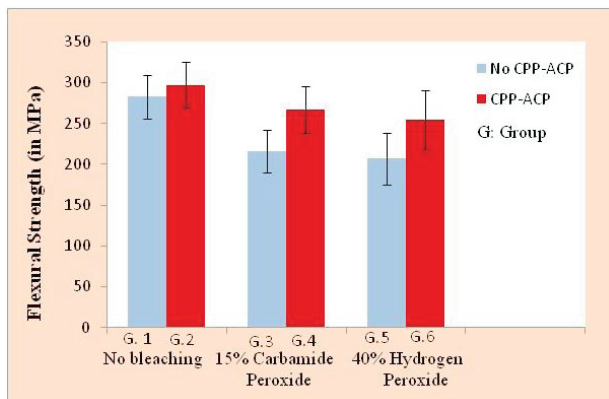


Figure 2. The mean and standard deviation of enamel-dentin flexural strength after application of bleaching agents and CPP-ACP (in MPa).

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, South Jourdan, UT, USA) for 1 hour/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°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) = $3P_f L / 2WH^2$, 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 occurred between bleaching and application of CPP-ACP ($p > 0.05$). Multiple comparisons by a post-hoc Tukey test revealed that all groups showed significant differences ($p < 0.05$), except between group 1 and 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 occurred 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 been associated with proteolytic reaction, which is due to induce denaturing 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 existence 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 occurred 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.

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