Effect of tooth bleaching agents with dicalcium phosphate dihydrate on the tooth whitening and enamel surface properties

Youn-Soo Shim

Department of Dentistry

The Graduate School, Yonsei University

Effect of tooth bleaching agents with dicalcium phosphate dihydrate on the tooth whitening and enamel surface properties

Directed by Professor Kwang-Mahn Kim, DDS., Ph.D.

The Doctoral Dissertation
submitted to the Department of Dentistry,
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Youn-Soo Shim

December 2009

This certifies that the Doctoral Dissertation of Youn-Soo Shim is approved.

Thesis Supervisor : Prof. Kwang-Mahn Kim

[Kyoung-Nam Kim: Thesis Committee Member #1]

[Yong-Keun Lee: Thesis Committee Member #2]

[Byoung-Duck Roh: Thesis Committee Member #3]

[June-Sung Shim: Thesis Committee Member #4]

The Graduate School
Yonsei University

December

감사의 글

제가 한참 논문을 정리할 때가 산에 단풍이 울긋불긋 예쁘게 물들 때였습니다. 지난 시간을 돌이켜 보니 제가 처음 치과생체재료공학교실에 들어와서, 공부하는 동안 저의 보물인 두 아이를 낳았고, 휴학을 하며 그렇게 4년이 흘렀습니다. 많이 힘들었던 시간이었지만 주위의 도움이 있었기에 가능한 일이었습니다.

물론 지도교수님이신 김광만 교수님의 지도와 독려가 가장 큰 힘이었습니다. 늘 한 결 같이 제자를 대해주시고 교실의 든든한 버팀목이신 교수님이십니다. 글의 구성이나 실험계획이 부족했던 저에게 바쁘신 와중에도 체계적이고 세심한 부분까지 신경 써 주신 것에 대해 진심으로 감사의 말씀 드립니다. 항상 용기와 긍정의 힘을 아끼지 않으셨던 김경남 교수님 감사드립니다. 그리고 논문에 쓰이는 재료의 특성과 관련된 서적까지 빌려주시면서 지도해 주신 이용근 교수님 감사드립니다. 진료에 바쁘신 중에도 부족한 부분에 많은 조언과 지도를 해주신 노병덕 교수님과 심준성 교수님께 감사드립니다.

학업과 일을 병행하며 할 수 있도록 지원해주신 부모님께 감사드립니다. 힘드시다는 말씀 없이 두 아이를 번갈아 돌봐주시고, 먹을 것까지 챙겨서 보내주시고 저의 뒤에서 항상 함께 하시며 사랑을 전해 주셨습니다. 그리고 남편과 두 아들은 저의 든든한 후원자였습니다. 음악에 맞춰 노래 부르며 춤을 추고, 볼에 뽀뽀해 주는 모습을 보면 입가에 웃음이 나면서 행복을 느낍니다. 엄마가 같이 놀아주지도 못하고, 책도 읽어 주지 못한 날이 많았는데도 투정 없이 잘 커준 아이들이 사랑스럽습니다. 주말이면 엄마 방해된다며 아이들을 데리고 나가며 저에게 외조를 아끼지 않은 남편과 손

주들의 재롱에 웃음지어 주시고, 배려해주신 시부모님께 감사드립니다.

교실을 드나드는 동안 많은 선생님들의 열심인 모습이 저에게 반성과 채찍을 다시금 할 수 있게 하는 동기부여가 됐습니다. 언제 어디서 만나도 항상 반갑고 힘이 되어준 친구이면서 언니인 장선옥 선생님, 이주혜 선생님, 항상 바쁘신 가운데도 성심껏 설명해주시고 가르쳐 주셨던 박정종 교수님과 이상배 박사님께 감사의 마음을 전합니다. 아기 엄마가 될 예쁜 제진아 선생님, 같이 심사를 준비하던 똑똑한 한미라 선생님, 환한 웃음이 예쁜 강민경 선생님, 든든한 입학 동기인 문승균 선생님, 졸업시험 준비하는 시은 선생님, 우리 과의 든든한 조교이신 김우현, 진강식 선생님 모두 좋은 인연으로 함께 한 시간이었습니다.

저보다 먼저 박사과정에 들어갔지만 아직 졸업 못하고 지금은 본인의 논문을 쓰면서 언니의 논문을 도와준 고마운 동생 명희에게 고마운 마음을 전합니다. 석사와 박사때의 논문 제본을 해주신 고모, 고모부님께 감사드립니다. 제가 병원 근무하는 동안 학업을 계속 할 수 있도록 지원해 주신 박영국 교수님, 서울대병원에서 만난 인연으로 통계자문을 성심으로 해주신 김혜영 교수님께 깊은 감사드립니다. 학교 선배님이시며 직장에서 많은 격려를 해주신 윤미숙 교수님, 편안한 성품을 지닌 김수경 교수님, 많은 힘과 배려를 아끼지 않으신 임순환, 권미영 교수님께 감사드립니다. 첫 직장의 인연으로 13년 동안 연락하며 도움을 아끼지 않으신 박경리 사무장님과 김숙희 선생님께 감사드립니다. 개원해서 환자들의 친절한 의사 선생님이며 친구인 최성호 선생님, 같이 박사과정을 마치게 되어 서로 힘이 된 소아치과 안소연 선생님, 석사동기이면서 절친 신의정, 잘생긴 아들을 낳아 엄마가 된 후배 정상희 선생님께 감사드립니다. 대학 동창으로 어디에 있어도 한결 같은 우정으로 서로의 힘이 되어준 친구 박수영, 김세란, 이현옥, 얼

마 전 아들 낳은 예쁜 이수영 선생님, 첫 시간강사를 같이 한 정미희 선생님, 항상 격려해 주고 힘을 준 한순옥 선생님 진심으로 감사드립니다. 해맑은 미소를 지으며 치아를 구해다 주고 교수님 논문 잘 쓰시라고 지원해 준 제자 박새롬, 바쁜 병원 생활 중에도 많은 도움을 주신 김순옥 선생님, 나를 따라 학회에 들어와서 힘든 일을 해도 내색 안 하고 애써 주신 조윤정 선생님께 감사의 마음을 전하며, 이 모든 분들이 있었기에 가능한 일이었습니다.

다른 사람들의 논문을 받고 감사의 글을 읽을 때, 내가 글을 쓸 때가 되면 누구누구 이름을 써야지 하며 글을 읽었던 때가 생각이 납니다. 막상 지금 글을 쓰려고 하니 머릿속에 정리가 제대로 안된 채 글을 쓰는 것 같네요. 두 아이가 잠든 틈을 타서 마지막 글을 적습니다.

이런 과정이 끝이 아니고 또 다른 도전의 시작이 될 것이라고 생각합니다. 앞으로 5년, 10년 후의 모습이 궁금해 주위의 사람들과 자주 얘기합니다.

그때의 저의 모습은 무엇보다 진정으로 늘 감사하며, 사랑하며, 행복한 사람으로 살기를 바라는 맘과 함께 글을 마무리 합니다.

2009년 12월

심 연수 드림

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Effect of tooth-bleaching agents with dicalcium phosphate dihydrate on the tooth whitening and enamel surface properties

Youn-Soo Shim

Department of Dentistry

The Graduate School, Yonsei University

(Directed by Professor Kwang-Mahn Kim, D.D.S., Ph.D.)

In recent year, there has been an increasing demand for tooth bleaching in order to improve the esthetic appearance of patients, because it is an easy, effective, and non-destructive treatment to remove these tooth discolorations. Hydrogen peroxide (HP) acts as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules, and HP anions. Current studies have presented contradictory results concerning the adverse effects of tooth bleaching agent on enamel surfaces.

The purpose of this study was to evaluate the tooth whitening and properties of an enamel surface after treatments with tooth bleaching agents that contained dicalcium phosphate dihydrate and HP. DCPD ($CaHPO_4 \cdot 2H_2O$) is known to be a precursor to hydroxyapatite (HA) in the remineralization process. Many studies have shown that supplemental calcium and phosphate

in the form of DCPD have an anticaries benefit. In acidic solutions, DCPD was thermodynamically more stable than other calcium phosphates. This study selected 35 % HP and 3.5 % HP, which were used in-office bleaching and home bleaching agent. Sixty enamel specimens were obtained from human premolars and randomly divided into two groups. These groups were then divided into three subgroups (n=10). Tooth bleaching agents were prepared with DCPD (0 g for controls, 0.1 g and 1 g for experimental groups) and HP solution (35 % and 3.5 % HP). In the Group A, DCPD / 35 % HP solutions were applied on sectioned enamel surfaces for 60 min per day for 1 day. In the Group B, DCPD / 3.5 % HP solutions were applied on sectioned enamel surfaces for 8 hours per day for 14 days. pH and mineral composition (ICP-AES) in the tooth bleaching agent were measured. Tooth color, microhardness and surface characterization (SEM and EDX) of enamel surface were measured as well.

1. The tooth bleaching agents with DCPD showed a significant increase in pH as compared with the ones without DCPD in the group A ($p<0.05$). In the group B, however, there was no statistically significant difference ($p>0.05$).

2. As the concentration of DCPD was increased, the concentration of Ca and P was also increased. The ratio of Ca/P was found to be approximately 1.1 ~ 1.2.

3. In all groups, after the tooth whitening, the tooth color was found to have a value of L^* which was significantly increased, while that of a^* and b^* were significantly decreased as compared with the ones that were seen prior to the tooth whitening ($p<0.05$).

4. In all groups, the hardness of tooth after bleaching showed a significant decrease in the microhardness as compared with the one prior to tooth bleaching ($p<0.05$). In both group A and B, however, as DCPD content was increased, the decreasing degree of microhardness was found to be smaller in the experimental group as compared with the control group.

5. Following an analysis of the characteristics of enamel surface after bleaching, there were porosity and erosion in the control group. In the groups where the amount of DCPD was the greatest, however, there was no surface change.

6. Following an analysis of the constituents of enamel surface after bleaching, as DCPD content was increased, the amount of Ca and P was significantly increased ($p<0.05$).

Based on the above results, tooth bleaching agents of 35 % and 3.5 % of HP containing DCPD are equally effective to the control group. By raising the pH and thereby effectively reducing the decalcification of tooth surface, a lower degree of the effects are given to the surface characteristics and constituent alterations of enamel. Thus, the commercial availability can be achieved for the constituents of tooth whitening materials.

Keywords: Tooth bleaching, DCPD, pH, Tooth color, Microhardness

Effect of tooth-bleaching agents with dicalcium phosphate dihydrate on the tooth whitening and enamel surface properties

Youn-Soo Shim

Department of Dentistry, The Graduate School,
Yonsei University

(Directed by Professor Kwang-Mahn Kim, D.D.S., Ph.D.)

Ⅰ. INTRODUCTION

1. History of tooth bleaching

For well over a century, bleaching has been used to achieve a lighter and more desirable tooth color. Tooth bleaching is not a new technique in dentistry.[1] By the mid-1800s, the bleaching agent of choice for nonvital teeth was chloride of lime.[2] As early as the 1890's, a 3 % solution of Pyrozone was used safely as a mouthwash by both children and adults by Atkinson. It reduced caries and whitened the teeth. By 1918, an in-office bleaching technique similar to technique used today, using high concentrations of hydrogen peroxide (HP) activated by heat or light, was well established. In 1937, Ames et al. reported a

technique using a mixture of HP and ethyl ether on cotton, heated with a metal instrument for 30 minutes, and applied over 5 to 25 visits to treat mottled enamel.[1] In 1961, Pyrozone and sodium perborate were used for nonvital teeth by Spasser.[2] In 1966, the combined use of hydrochloric acid and HP was promoted to remove brown stain from mottled teeth by McInnes. In 1967, the walking bleaching technique uses 35 % HP and sodium perborate for whitening nonvital teeth by Poe et al.[1] In 1968, the current home bleaching technique, employing a custom-fit tray containing 10 % carbamide peroxide (CP) solution, was first used by Klusmeier. In 1970, Cohen and Parkins published a method for whitening tetracycline-discolored dentine of the teeth of young adults treated for cystic fibrosis.[1] The first commercially available 10 % CP was developed and subsequently marketed by Omni International in 1989 based on the findings of Munro, who used a 10 % CP solution to control inflammation after root planning in a vacuum-formed plastic splint. Haywood et al. published the first clinical study on tooth whitening using Proxigel in vacuum-formed custom trays.[1] This is the technique known as `night guard vital bleaching' now commonly used.

Various chemical combinations have been tried. Currently available peroxide-containing tooth-whitening materials include professionally dispensed and supervised products for use by patients at home, professional-use in-office products, and over-the-counter products for sale directly to consumers.

2. Tooth discolorations / Stains

The color of teeth is primary determined by the dentin but is influenced by the color, translucency and varying degrees of calcification of enamel as well as its thickness, which is notably greatest at the occlusal or incisal edge. The normal color of teeth is determined by the blue, green and pink tints of enamel and is reinforced by the yellow through to brown shades of the dentin beneath.[3] Many types of color problems affect the appearance of the teeth. The cause of these problems varies, so the speed with which they may be removed also varies.

Discolorations are classified as extrinsic or intrinsic. Extrinsic discoloration arises when external chromogens are deposited on the tooth surface or within the pellicle layer. Intrinsic discoloration occurs when the chromogens are deposited within the bulk of the teeth (usually in the dentin) and are often of systemic or pulpal origin.[4] Some extrinsic stains that remain on the tooth for a long time become intrinsic.[2]

Extrinsic color changes may be due to poor oral hygiene, ingestion of chromatogenic foods and drinks, and tobacco use. Intrinsic color changes may be caused by aging, ingestion of chromatogenic foods and drinks, tobacco usage, microcracks in the enamel, tetracycline medication, excessive fluoride ingestion, severe jaundice in infancy, porphyria, dental caries, restorations, and the thinning of the enamel layer.[3] Other, less common, medical situations and conditions may also cause the loss of desirable tooth color. The causes of staining need to be assessed carefully to better predict the rate and degree to which bleaching will improve tooth color, since some stains are more responsive to the process than others.[4,5]

3. Mechanism of tooth bleaching

Contemporary bleaching agents are typically either hydrogen peroxide (HP), carbamide peroxide (CP), sodium percarbonate, or some other compound.[3] HP is a main component of tooth bleaching. CP is a chemical adduct of urea and HP, which upon dissolving in water or saliva disassociates back into HP and urea. CP and HP penetrate the enamel and dentin, reaching the pulp in 5 to 15 minutes. It is not only the enamel but mainly the dentin that chamber color, and it does next to the dentinoenamel junction.[6] HP is a very pale blue liquid, slightly more viscous than water, that appears colorless in dilute solution. It is a weak acid, has strong oxidizing properties, and is a powerful bleaching agent. It is used as a disinfectant, antiseptic, oxidizer, and in rocketry as a propellant.[5] The oxidizing capacity of HP is so strong that it is considered a highly reactive oxygen species. HP always decomposes (disproportionates) exothermically into water and oxygen gas spontaneously:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

HP acts as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules and HP anions.[7] These reduce or cleave pigment molecule double bonds to either break down pigments to small enough molecules that diffuse out of the tooth or to those that absorb less light and hence appear lighter. Such pigmented molecules tend to be organic, although inorganic molecules can be affected these reactions (Figure 1).[3] Under photochemically initiated reactions using light or lasers, the formation of hydroxyl radicals from HP has been shown increase.[8]

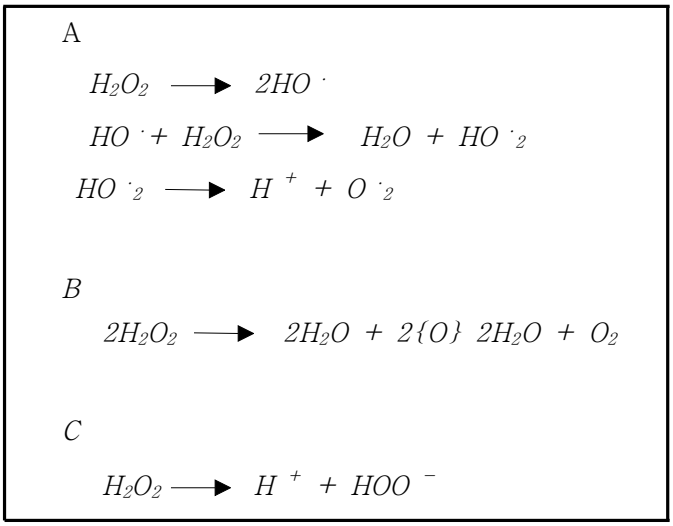

Figure 1. Hydrogen peroxide forms free radicals like hydroxyl and perhydroxyl radicals and superoxide anions (A), reactive oxygen molecules that are unstable and transformed to oxygen (B), and hydrogen peroxide anions (C).[3]

4. Tooth bleaching systems

4.1. Non-vital bleaching

Non-vital bleaching techniques include an in-office thermocatalytic technique and an out of the office technique referred to as walking bleach. It is usually performed on traumatized discolored teeth and only after a root canal treatment. The thermocatalytic technique uses heat applied several times during a 30-minute period to activate the solution in the pulp chamber, after which the solution is rinsed from the chamber.[9] Walking bleach uses a mixture of 30 % HP and perborate to make a paste that is sealed into the chamber to permit activation of the solution over several days. Although non-vital bleaching is quite effective, there is a slight potential for a most deleterious side effect of cervical resorption. Prior to any bleaching procedure the nonvital tooth must be radiographed to assess the quality of the root canal obturation and the apical tissue.

4.2. Vital bleaching

4.2.1. In-office or power bleaching

In-office bleaching of vital teeth generally uses high concentration (15 ~ 30 %) HP solution placed directly on the teeth and may involve application of light and/or heat to activate or enhance the peroxide release.[10] Advantages were included minimal dependence on patient compliance and immediate visible results, which satisfy patients who to see quick results. The disadvantages are higher patient cost, the use of chair time, and the requirement of multiple in-office

visits to get optimal results and retain them.

4.2.2. Dentist-supervised at home bleaching

It is performed using custom made mouth trays fitted by the dentist using lower concentrations of HP (2 ~ 10 %) and applied for several hours a day for several weeks. The benefits achieved with tray-based system are widely known ; lower incidence of tooth sensitivity or gingival irritation; less visits to the dental office; achievement of the same whitening results as higher concentrations agents; and safety and efficacy of 10 % CP that has already been established in published clinical trials.[11] The major disadvantage is the need for in-office chair time and the potential fir soft-tissue changes with excessively extended use. Also, this treatment is not a one-time treatment, which patients prefer.

4.2.3. Over-the-counter (OTC) bleaching

Over-the-counter products include dentifrices, whitening strips, paint-on brush applications, and whitening kits complete with a preformed or semimolded tray. Contact time is significantly reduced as compared with professionally prescribed products. Therefore, whitening strips and paint-on brush applications must be used longer to obtain similar results to those from the professionally prescribed products. Minimal research exists on these products because they can be bough and used indiscriminately by patients, the risk of inappropriate use is high. These methods are excellent for maintaining already whitened teeth and are a good option for patients who cannot afford the professionally prescribed products or who do not have time for multiple office visits.[12]

5. Effect of tooth bleaching

5.1. Efficacy

Night guard vital bleaching by 10 % CP has been in use for close to 20 years and has been the most extensively researched method for tooth whitening. Similarly, power bleaching using 35 % HP, with or without light and / or heat activation, has also been shown to be effective. It has been shown to be effective for lightening primary teeth discolored by trauma; lightening tetracycline-stained teeth; removing brown stain, including fluorosis stain; and lightening teeth stained by nicotine.[13,14] The teeth get lighter through the process and reach a maximum lightness regardless of the concentration of the agent or contact time used. Retention studies reported satisfactory shade retention in 82 % of the cases treated at 47 months posttreatment[15] and long-term results of bleaching efficacy were reported that by leonard,[16] with at least 43 % shade retention without retreatment at 10 years.

5.2. Side effect

5.2.1. Tooth & oral tissues

The most common side effect associated with tooth bleaching is tooth sensitivity. Short-term pulpal response varies from patient to patient and even from tooth to tooth. Double-blind studies have reported sensitivity in 25 % to 70 % of patients. Sensitivity is in the form of a reversible pulpitis caused from the dentinal fluid flow and pulpal contact of the material without apparent harm to the pulp.[17] These chemicals may change the osmolarity of the fluids in the pulp and dentin, producing a reversible pulpitis. Peroxide does penetrate through the tooth the pulp in a matter of minutes.[18] Therefore, it can produce sensitivity, but the pulp remains healthy and the sensitivity is completely reversible.[19] It is import that the process be carefully monitored to avoid creating great sensitivity in the teeth. Cervical resorption is seen occasionally in bleached, root-filled teeth and has been attributed to a combination of trauma and bleaching with high concentration HP and heat.

5.2.2. Mechanical and chemical properties of enamel and dentin

Another consideration with tooth bleaching is the effect it has on enamel and dentin. As the bleaching process often involves direct contact of the bleaching product with the surface of the teeth for an extended period of time, many studies have evaluated the effects of peroxide-based products on the physical and chemical properties of tooth enamel and dentin. Changes of the surface texture after application of HP and CP bleaching gels are described as surface

morphology, decalcification, chemical composition, surface-softening lesions, or surface roughness some also found a reduction in microhardness in enamel and dentin.[20-24] Studies evaluating the effects of home bleaching products on restorative materials have also produced equivocal results. Bleaching does interfere with the bonding process because it results in the presence of HP in the enamel and dentin, which hinders polymerization of the resin composite.[25,26] Resin tags in bleached enamel are less numerous, less well defined and shorter than those in unbleached enamel.[25,26]

6. Toxic effects

CP administrated as a home bleaching agent breaks down to produce hydrogen peroxide and urea in the mouth. The toxicology of HP has been reported that HP is found to occur naturally in many vegetables and is produced in humans during normal metabolism of aerobic cells.[27] The study reported that the average amount of tooth bleaching agent used per application is 502 mg. All of this amount swallowed would not exceed 8.37 mg / kg,[27] which is below the 10 mg / kg associated with acute toxicity in rats.[28] The dermal toxicity of HP is low, with concentrations of up to 35 % not considered as being irritant to rabbit skin; however, concentrations of greater than 50 % are corrosive.[38] Various mouth rinses and oral antiseptics (10 and 15 % CP), and HP up to a concentration of (3 %) have been used for some time with no detectable ill effect on humans and have been approved by the US Food and Drug Administration since 1979. On the contrary, reports are of beneficial effects on gingival irritation and plaque accumulation, and an anticariogenic action.[29] HP has been found not to be carcinogenic, mutagenic or teratogenic,[30] and concerns of toxicity or serious damage to soft tissues appear to be unfounded.[31]

7. Indications and contraindications for bleaching

Nearly every patient can have their teeth bleached, but not every case is guaranteed to have a successful outcome or be enough to satisfy the esthetic needs of the patient. The indications for bleaching are basically the same for both in-office and home bleaching but the clinician must decide which method is best suited to the patient's needs.[3]

7.1. Indications for bleaching

Generalized staining (smoking dietary stains such as those of tea and coffee) may be completely removed by bleaching. Bleaching may be done in lieu of bonded resin composite restorations, porcelain veneers, or crowns to improve the tooth color. Patients may be satisfied with the results of bleaching such that more invasive treatment is not needed. Very severe tetracycline staining may not be amenable to bleaching alone and therefore combination treatments, such as bleaching and veneers, may be considered. Fluorosis with multiple spots of varying colors may require a combination of bleaching and microabrasion using hydrochloric acid and abrasives / polishes.[32] Bleaching can also be used post-restoratively when patients present with incorrectly made restorations whose shades are lighter than the natural dentition.

7.2. Contraindications for bleaching

Although bleaching is a safe and effective aid in improving the appearance of the teeth, not every discolored tooth requires bleaching. Patients with

hypersensitive teeth are generally not good candidates for bleaching, although management of the sensitivity may allow successful bleaching of their bleaching.

Contraindications for in-office bleaching with high concentrations of HP include teeth with extremely large pulps, exposed root surfaces, or severe enamel loss. At-home bleaching is generally not indicated for pregnant women or patients allergic to the ingredients in the CP preparations.[33] Patients with a history of temporomandibular disorder (TMD) may not be good candidates for at-home bleaching or may need to wear the tray during waking hours only.[34]

All other delineators, such as pulp size, exposed dentin, cracks, gingival recession, caries, gender or age of the patient, or other physical characteristics, were not predictive of tooth sensitivity. In the case of decay, a restoration can be preformed and resurfaced for the correct bleached shade about 2 weeks post-bleaching once the end shade has stabilized and to allow for the dissipation of the residual oxygen that may inhibit the composite bond to enamel / dentin.[25] Patients with existing esthetic restorations must be warned that when bleaching lightens the natural tooth color, restorations may appear relatively dark and unattractive. The need for new restorations that are lighter in shade should be discussed with the patient prior to bleaching.[3]

8. Dicalcium phosphate dihydrate (DCPD)

Dicalcium Phosphate Dihydrate (DCPD), also known as Brushite, is a hydrated calcium phosphate mineral with the composition $CaHPO_4 \cdot 2H_2O$.

It is practically insoluble in water, with a solubility of 0.02 g per 100 mL at 25 °C. It contains about 23 percent calcium in its anyhydrous form, and is mainly used as a dietary supplement in prepared breakfast cereals, dog treats, enriched flour, and noodle products. It is also used as a tableting agent in some pharmaceutical preparations. It is used in poultry feed. The structure of DCPD is almost identical to that of gypsum with their respective unit cell dimensions very similar. The phosphate of DCPD is isomorphous with arsenate, which forms instead, the mineral pharmacolite.[35]

The backbone of DCPD is made up of alternating calcium and phosphorus atoms which form chains via the sharing of oxygens (the four phosphate oxygens, Figure. 2). The phosphorus atoms have four fold coordination forming phosphate tetrahedra while the calcium atoms have eight fold coordination. Four of calcium's bonds are with these phosphate tetrahedra, forming the backbone chains, while two more of the calcium bonds are with oxygens from neighboring chains. These last bonds which link the chains together result in a zig-zag pattern or corrugated sheet. The last two calcium bonds are with the water molecules. These water molecules are what hold the sheets together.[35]

Calcium and phosphorus make up the majority of an animals mineral nutrient requirements (to fulfill both body tissue and milk needs), therefore DCPD is a common and widely used animal supplement. DCPD is also of interest because it is the most soluble of the sparingly soluble calcium phosphate minerals and thus makes a good candidate for rock phosphate dissolution studies. The fate of DCPD in soils is rather transient. Typically, mineral phosphorus is added to soil in a

water soluble form such as triple superphosphate or diammonium phosphate. As the phosphorus dissolves the high solution P concentration favors precipitation reactions. In neutral and calcareous soils, DCPD is one of the first reaction products.[35]

DCPD is an acidic calcium phosphate phase which can form from tooth mineral under caries-like conditions.[36] DCPD has been known as a precursor phase to HA in the remineralization process, and also as one of the constituents found in dental calculus and precarious lesions. Therefore, DCPD often coexists with HA in the dental hard tissues and interacts with saliva in the oral cavity at the same time.[37] Previous clinical trials have shown that supplemental calcium and phosphate in the form of DCPD have an anticaries benefit. It has been shown that DCPD incorporated into snacks[38] and chewing gums[39] reduced the incidence of caries in children. Dentifrices containing DCPD as the abrasive are very popular and are sold throughout the world. There is evidence from intra-oral remineralization studies that indicate that DCPD works with fluoride to provide improved remineralization benefit above and beyond that of fluoride alone. For DCPD to work with fluoride, DCPD must be hydrolyzed in the oral environment to release calcium and phosphate ions. The use of saturated DCPD in remineralization of artificial caries lesions in vitro.[40]

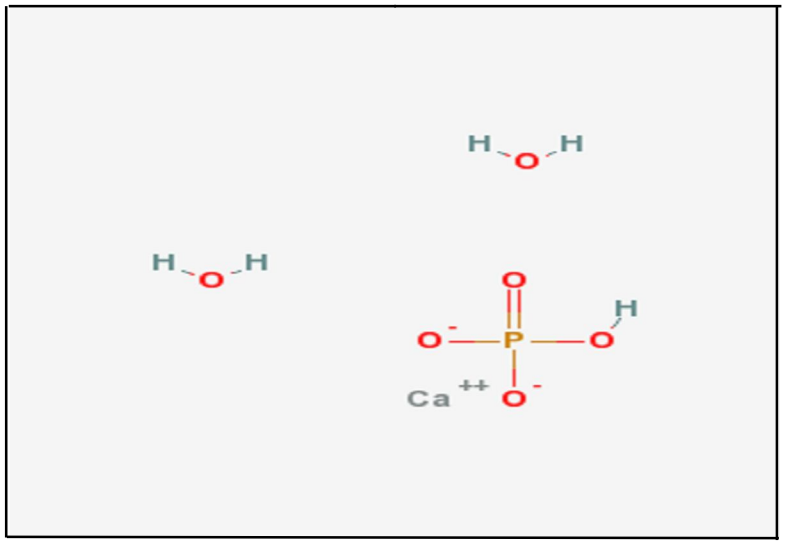

Figure 2. The structure formula of DCPD.

9. Research objectives and scope

Recently, there has been an increased demand for tooth bleaching in order to improve the esthetic appearance of patients, because it is an easy, conservative method. Bleaching agents cause lightening of discolored tooth structures through the decomposition of peroxides into free radicals. HP is a main component of tooth bleaching. CP is a chemical adduct of urea and HP, which upon dissolving in water or saliva disassociates back into HP and urea. The 10 % CP, upon contact with oral tissues and saliva, breaks down into 3 % to 3.5 % HP and 6.5 % to 7 % urea.[2] 35 % HP and 10 % CP is the most commonly used in-office bleaching and home bleaching agent. As the whitening process often involves direct contact of the whitening product with the surface of the teeth for an extended period of time, numerous studies have evaluated the effects of products that contain peroxide on the physical and chemical properties of tooth enamel.[22] Some studies found that tooth bleaching led to tooth sensitivity or changes in chemical composition, surface morphology, or surface roughness some also found a reduction in microhardness.[20-24] On the other hand, several studies did not find any significant changes in the surface properties of tooth enamel.[41-43] Therefore, the effect of bleaching treatment on dental enamel is not yet clear and remains controversial. Although, no clinical studies or case reports in the literature have documented macroscopically or clinically visible demage due to vital bleaching or clinically relevant tissue destruction, It is important to minimize the risk of even minor damage in order to ensure life-long integrity of dental hard tissue.[44]

To minimize damage, ingredients like fluoride, calcium, sodium nitrate, CPP-ACP, or synthetic hydroxyapatite (HA) have been added to the bleaching agent by the manufactures. However, the final results may be unpredictable.[45]

Dicalcium phosphate dihydrate (DCPD) is known to be a precursor to HA in the remineralization process. Therefore, DCPD often coexists with HA in the hard dental tissues and interacts with saliva in the oral cavity.[38] In acidic solutions, DCPD was thermodynamically more stable than other calcium phosphates. On the other hand, in neutral and basic solutions, other calcium phosphates were more stable.[46] Thus, DCPD was suited to ingredient of tooth bleaching agent.

The effects of DCPD / HP agents with different pH on the enamel surface microhardness, morphology, composition, as well as their potential for affecting tooth color change, have also been questioned.

The aim of this study was to evaluate the effects of HP bleaching agents that contain DCPD on the tooth whitening and enamel surface properties. The null hypothesis that tooth bleaching agents with and without DCPD were no difference the tooth whitening and enamel surface properties.

Ⅱ. MATERIALS AND METHODS

1. Materials

1.1. Hydrogen Peroxide (HP)

This study was used 35 % Hydrogen peroxide (Sigma-Aldrich, USA).

1.2. Dicalcium phosphate dehydrate (DCPD)

This study was used dicalcium phosphate dehydrate (Junsei Chemical Co. Ltd, Japan) powder.

2. Methods

2.1. Tooth bleaching agents containing DCPD

The 35 % HP solution was diluted with distilled water to obtain a 3.5 % solution. The tooth bleaching agents were prepared by adding 0 g for controls, 0.1 and 1 g of DCPD powder to 100 ml each of either 35 % HP (Group A) or 3.5 % HP (Group B) solution (Table 1). The DCPD / HP agents were stirred using a magnetic stir-bar at room temperature for 24 hours. In the pilot study, surface properties were no difference on concentration over the 1 g DCPD. Thus, this study selected 0.1 and 1 g of DCPD concentrations.

Table 1. The composition of bleaching agent containing DCPD

Group	Subgroup (n=10)	35 % HP (100 ml)	Distilled water (100 ml)	DCPD (g)
A	A1	100	0	0
	A2	100	0	0.1
	A3	100	0	1
B	B1	10	90	0
	B2	10	90	0.1
	B3	10	90	1

2.2. pH-measurements

After stirring, the pH value of each solution was measured with an pH meter (Thermo Fisher Scientific, USA).

2.3. Mineral measurements

An inductively coupled plasma-atomic emission spectrometer (ICP-AES, OPTIMA 4300DV, Perkin-Elmer, USA) was used for quantitative analysis of the elements dissolved in the DCPD / HP agents.

2.4. Preparation of the specimens

The study protocol was reviewed and approved by the Ethics Committee of the Dental Hospital of Yonsei University College of Dentistry. Fifty human premolars, extracted for orthodontic reasons, were stored in distilled water. Teeth exhibiting any visible cracks, dentin exposures or hypoplastic defects were excluded. The enamel surfaces were then cleaned with a prophylaxis paste to remove any extrinsic stain.

The roots were removed and immersed into a black tea solution using the method described by Sulieman and others.[47] This method result in a combination of both extrinsic and intrinsic staining, the majority of which is intrinsic. A black tea solution was produced by boiling 2 g of tea (Marks and Spencer Tea Extra Strong, Marks and Spencer, UK) in 100 ml of distilled water for ten minutes and filtered through gauze to remove the tea leaves from the infusion. The enamel

specimens were immersed in tea for 24 hours. After staining was complete, the tooth were rinsed with distilled water.

Each tooth was sectioned into 2 fragments (4mm×4mm×3mm) with a low-speed saw (NTI-kahla GmbH, Germany). The 60 enamel specimens selected microhardness value between 300 and 350 VHN. The enamel surfaces of the specimens were ground flat and polished using a polishing machine (Polisher DP-1, Dae Heung Science, KOREA) with 600, 1200, 1800, 2400 and 4000 grits silicon carbide papers (ALLIED, High tech products. Inc, CA). The polished tooth were cleaned ultrasonically in distilled water for 5 minutes. The specimens were embedded in plastic moulds with the enamel surface exposed for bleaching (Figure 3).

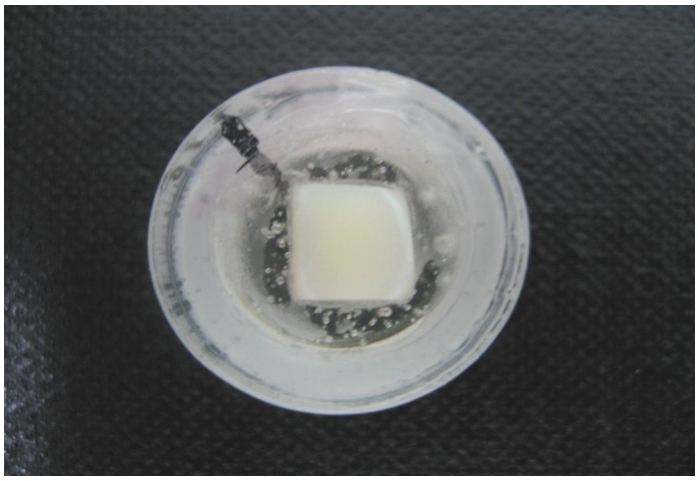

Figure 3. Tooth specimen embedded in plastic mould.

2.5. Bleaching procedure

The resulting 60 specimens were randomly divided into two groups (n=30 per group, Table 1). Each of these groups were subdivided into three subgroups (n=10 per subgroup). In the Group A, each subgroup received applications of 0, 0.1, and 1 g DCPD combined with 35 % HP on sectioned enamel surfaces for 15 min a day. The bleaching procedure was repeated 4 times so that the total application time was 1 hour. After bleaching, the enamel surfaces were rinsed under distilled running water for 30 seconds. In the Group B, each subgroup received applications of 0, 0.1, and 1 g DCPD combined with 3.5 % HP on sectioned enamel surfaces for 8 hours per day for 14 days. After eight hours, the enamel surfaces were rinsed under distilled running water for 30 seconds. During the remaining time (16 hours per day), the specimens were maintained in individual vials filled with 1 ml of distilled water at 37 °C. Distilled water was replaced every day. Enamel changes were evaluated in four aspects: the color change, microhardness, SEM and EDX (Figure 4).

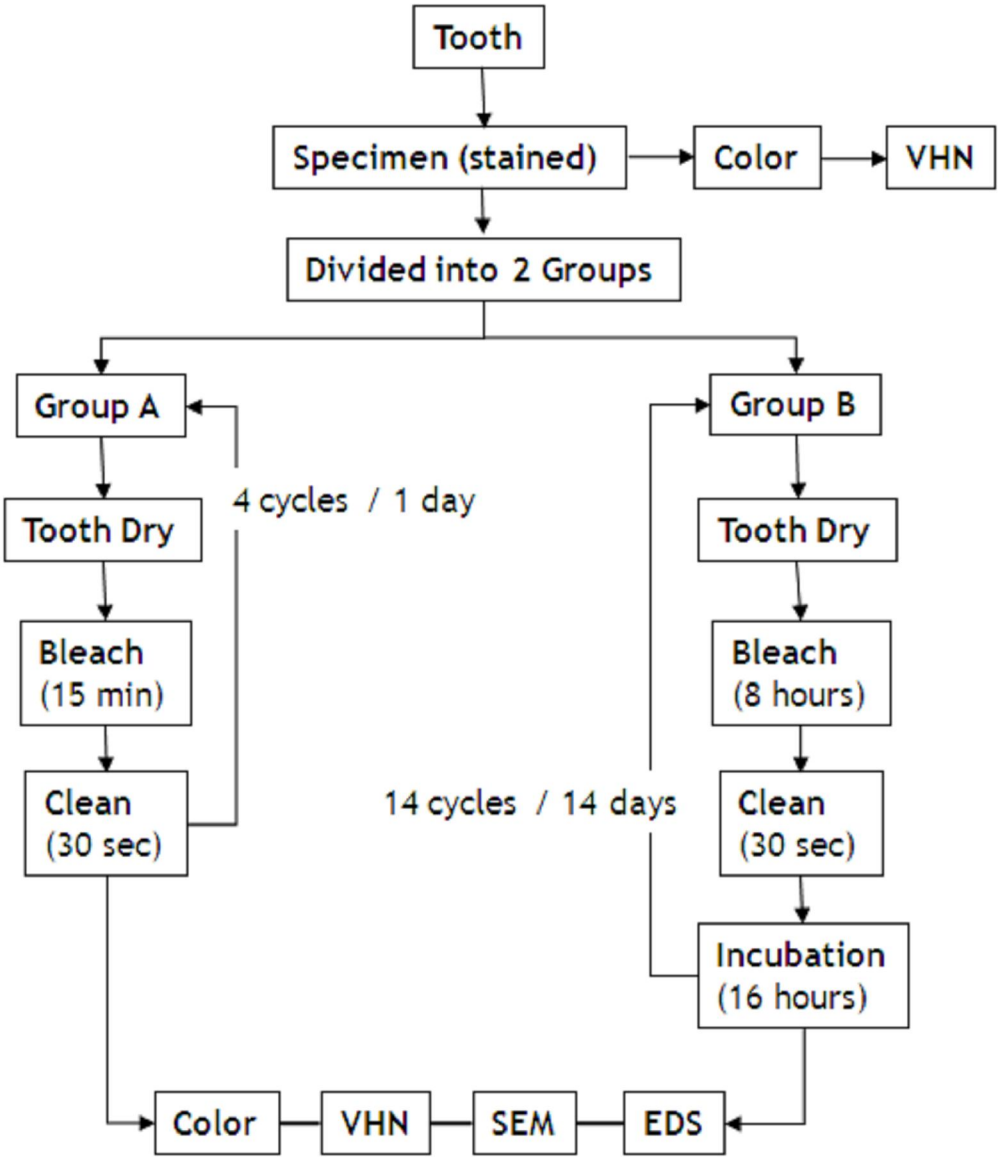

Figure 4. Flowchart of the study procedures and measurements.

2.6. Color measurements

Color measurements were carried out before and after bleaching with a spectrophotometer (MINOLTA CM-3500d, Japan) in the L^* a^* b^* mode described by the Commission Internationale de l'Eclairage (International Commission on Illumination, CIELab). In this mode, the L^* represents the light value (brightness), the a^* represents either green ($-$ a^*) or red ($+$ a^*), and the b^* represents either blue ($-$ b^*) or yellow ($+$ b^*). Prior to the measurements, the spectrophotometer was calibrated with white and black reflectance standards supplied by the manufacturer. The L^* a^* b^* values were estimated from the middle of the buccal side of each tooth. The difference between the color coordinates was calculated as : $\triangle E = \{(\triangle L^*)^2 + (\triangle a^*)^2 + (\triangle b^*)^2\}^{1/2}$

2.7. Microhardness measurements

Each specimen from the distilled water was dried in air. Microhardness was measured on a hardness testing machine (Micro-hardness tester, Japan) with a load of 200 g for 10 s intervals. The mean of four indentations was calculated. Before measurements, the enamel surface of the specimen was wiped to remove moisture. The average baseline microhardness was designated VHN(B), and the average after microhardness was designated VHN(A). The percentage microhardness loss (PML) was calculated with the following calculation:

$$PML(\%) = \frac{VHN(B) - VHN(A)}{VHN(B)} \times 100$$

2.8. SEM observation

To examine the changes in surface morphology after bleaching, the specimens were selected and cleaned ultrasonically in distilled water. Dried teeth were gold sputter-coated and examined with a field-emission scanning electron microscope (FE-SEM, S-4700, Hitachi, Japan).

2.9. EDX analysis

Enamel surface concentrations of calcium and phosphorus were measured with energy dispersive X-ray spectroscopy (EDX, EMAX-350, Hitachi, Japan).

2.10. Statistical analysis

The data from pH, color, microhardness and EDX measurements were analyzed using a paired t- test, t- test and one-way ANOVA. Tukey's test was used for the post hoc analysis ($p < 0.05$ and 95 % confidence level were considered significant).

Ⅲ. RESULTS

3.1. pH-measurements

The pH of bleaching agents was shown in Table 2. The most acid pH value of all bleaching agents tested was that of the group A1 (2.4), while the most basic agent was the group B3 (6.9). With increasing DCPD concentration, the pH values in the agents increased, making it less acidic. It can be seen that a change in pH from 2.4 to 4.8, 6.4 to 6.9 causes a pronounced increase in the concentration of DCPD. The tooth bleaching agents containing DCPD had a statistically significant difference in pH as compared with those containing no DCPD in the group A ($p<0.05$). In the group B, however, there was no statistically significant difference ($p>0.05$).

Table 2. The pH of bleaching agents

Group	pH	p
A1	2.4^{a}	
A2	4.7^{b}	<0.05
A3	4.8^{b}	
B1	6.4	
B2	6.8	>0.05
B3	6.9	

p-values are determined by one-way ANOVA.

3.2. Mineral measurements

The dissolved Ca and P ions in HP solutions were shown in Table 3. The dissolved Ca and P ions increased in HP solutions and were also enhanced by increasing the concentration of DCPD. The molar ratios of Ca and P ions from the DCPD were approximately 1.1 ~ 1.2. The Ca ions was more prominent than the P ions with all DCPD / HP agents.

Table 3. Concentrations of minerals in bleaching agents (ppm)

Group	Ca	P
A1	0	0
A2	275.3	244.9
A3	2676.7	2096.5
B1	0	0
B2	252.1	208.7
B3	2419.7	2150.1

3.3. Color measurements

The L^*, a^*, b^* and △E values for the six groups are shown in Table 4, 5, 6 and 7. There was no significant difference on L^*, a^* and b^* values among the six groups at the baseline ($p>0.05$). In the final bleaching, the a^* value of Group A and B showed significant difference ($p<0.05$) except of the L^* and b^* value. Paired t-tests showed significant difference in color values of enamel before and after bleaching in all the groups ($p<0.05$). Results of the study from CIELab color parameters are shown : improvement of lightness (△L^*), reduction of redness (△a^*), reduction of yellowness (△b^*), overall color change (△E).

Comparison of the L^*, a^* and b^* values in six bleaching groups from baseline to 1 day revealed showed obvious changes. Mean changes of △L^*, △a^* and △b^* according to HP concentration are shown in Table 8. Comparisons between the groups showed significantly different values for means in L^* ($p<0.05$), while others parameters were not significant different ($p>0.05$).

Thus, there was no significant difference between control groups and DCPD / HP groups.

Table 4. Mean of L* values baseline and after bleaching treatments

	L*				
Group	baseline	1 day	7 days	14 days	P[1]
A1	49.0 ± 1.0	60.4 ± 0.6			0.000
A2	50.1 ± 1.3	61.3 ± 0.8			0.000
A3	48.7 ± 2.2	60.3 ± 1.9			0.000
P[2]	0.437	0.434			
B1	50.3 ± 2.0	57.1 ± 0.7	57.8 ± 0.8	60.6 ± 0.4	0.000
B2	51.4 ± 0.7	53.5 ± 0.7	56.2 ± 2.3	59.9 ± 1.0	0.000
B3	49.7 ± 1.6	55.1 ± 2.7	57.7 ± 2.6	60.6 ± 0.8	0.000
P[3]	0.445	0.432	0.462	0.428	

Values are reported as the Mean ± Standard deviation.

[1]p-values are determined by paired t-test and denote the significance between baseline and final bleaching.

[2]p-values are determined by one-way ANOVA and denote the significance among A1, A2 and A3 groups.

[3]p-values are determined by one-way ANOVA and denote the significance among B1, B2 and B3 groups.

Table 5. Mean of a* values baseline and after bleaching treatments

	a*				
Group	baseline	1 day	7 days	14 days	p[1]
A1	−0.5 ± 1.0	−1.2 ± 1.3[a]			0.012
A2	−0.1 ± 1.4	−0.5 ± 1.8[b]			0.025
A3	0.2 ± 1.2	−0.9 ± 1.7[b]			0.014
p[2]	0.241	0.018			
B1	0.2 ± 1.6	−2.6 ± 0.2[a]	−2.3 ± 0.2[a]	−2.9 ± 0.1[a]	0.000
B2	0.1 ± 0.2	−1.4 ± 0.6[b]	−2.1 ± 0.4[ab]	−2.5 ± 0.2[ab]	0.001
B3	0.1 ± 0.2	−1.6 ± 0.4[b]	−0.6 ± 2.0[b]	−2.1 ± 0.4[ab]	0.000
p[3]	0.450	0.025	0.020	0.022	

Values are reported as the Mean ± Standard deviation.

[1]p−values are determined by paired t−test and denote the significance between baseline and final bleaching.

[2]p−values are determined by one−way ANOVA and denote the significance among A1, A2 and A3 groups.

[3]p−values are determined by one−way ANOVA and denote the significance among B1, B2 and B3 groups.

Table 6. Mean of b* values baseline and after bleaching treatments

Group	b*				
	baseline	1 day	7 days	14 days	p[1]
A1	4.0 ± 0.9	−0.3 ± 1.8			0.009
A2	3.2 ± 0.8	−0.6 ± 2.3			0.034
A3	5.1 ± 0.7	−1.5 ± 1.7			0.005
p[2]	0.053	0.061			
B1	5.1 ± 1.1	−1.6 ± 0.2[a]	−1.4 ± 0.1[a]	−1.6 ± 0.2	0.000
B2	4.4 ± 0.5	−0.2 ± 0.9[b]	−0.1 ± 0.7[b]	−0.2 ± 0.3	0.000
B3	5.0 ± 1.0	−0.2 ± 0.8[b]	−1.0 ± 0.4[a]	−0.2 ± 0.5	0.000
p[3]	0.082	0.008	0.002	0.361	

Values are reported as the Mean ± Standard deviation.

[1]p−values are determined by paired t−test and denote the significance between baseline and final bleaching.

[2]p−values are determined by one−way ANOVA and denote the significance among A1, A2 and A3 groups.

[3]p−values are determined by one−way ANOVA and denote the significance among B1, B2 and B3 groups.

Table 7. Mean of △E values after bleaching treatments

Group	△E		
	1 day	7 days	14 days
A1	10.7 ± 1.0		
A2	10.8 ± 1.1		
A3	11.2 ± 0.7		
p[1]	0.061		
B1	9.5 ± 1.0[b]	9.7 ± 1.3[b]	12.3 ± 1.5[b]
B2	4.9 ± 7.1[a]	6.4 ± 1.7[a]	9.9 ± 0.6[a]
B3	9.1 ± 2.5[b]	9.4 ± 1.2[b]	11.6 ± 0.6[b]
p[2]	0.001	0.008	0.001

Values are reported as the Mean ± Standard deviation.

[1]p-values are determined by one-way ANOVA and denote the significance among A1, A2 and A3 groups.

[2]p-values are determined by one-way ANOVA and denote the significance among B1, B2 and B3 groups.

Table 8. Mean changes of △L*, △a* and △b* according to HP concentration

Group	△L*	p[1]	△a*	p[2]	△b*	p[3]
A1	11.4	0.000	−0.7	0.068	−4.37	0.170
B1	10.3		−3.1		−7.0	
A2	11.2	0.000	−0.4	0.320	−3.8	0.692
B2	8.5		−2.6		−4.6	
A3	11.6	0.005	−1.1	0.363	−6.6	0.171
B3	10.9		−2.2		−5.2	

p−values were determined by t−test.

[1]Denote the significance between Group A and B of the △L* values ($p<0.05$).

[2]Denote the significance between Group A and B of the △a* values ($p<0.05$).

[3]Denote the significance between Group A and B of the △b* values ($p<0.05$).

3.4. Microhardness measurements

The average microhardness and standard deviation values for enamel before and after treatment with DCPD / HP solutions are in Table 9, 10 and Figure 5. There was no statistically significant difference among the groups at baseline ($p>0.05$). Paired t-test showed significant difference ($p<0.05$) in mean microhardness values of enamel before and final bleaching in all the groups.

However, the DCPD concentration increased in the bleaching agents, microhardness values less decreased (A3, B3). Group A1 showed the lowest microhardness values (287.3 VHN) than that of Groups A2, A3, B1, B2 and B3.

In Group A, on the 1 day was statistical difference of microhardness values ($p<0.05$). In Group B, statistical difference of microhardness were verified baseline, 7 days, 14 days (Table 9). On the 1 day, microhardness values were similarly decreased at the Groups B1, B2 and B3. However, on the 7th and 14th days were statistical difference of microhardness values ($p<0.05$).

The PML of six groups are shown in Table 10. Statistical analyses showed that in Group A1 and B1, A2 and B2 microhardness values were significant difference ($p=0.000$), while Groups A3 and B3 revealed no significant difference ($p=0.720$).

Table 9. Mean values (SD) of baseline and after surface microhardness measurements for the six groups

Group	baseline	1day	7days	14 days	p[1]
A1	329.8 ± 6.9	287.3 ± 5.4[a]			0.000
A2	331.2 ± 3.7	305.9 ± 6.9[b]			0.000
A3	332.3 ± 3.8	316.9 ± 8.1[c]			0.000
p[2]	0.387	0.000			
B1	331.6 ± 5.6	322.1 ± 6.2	304.9 ± 5.1[a]	293.7 ± 7.2[a]	0.000
B2	329.8 ± 3.7	324.1 ± 4.6	316.7 ± 4.9[b]	301.5 ± 6.4[b]	0.000
B3	324.6 ± 6.5	318.1 ± 7.2	312.0 ± 6.4[b]	307.7 ± 5.3[b]	0.000
p[3]	0.225	0.102	0.009	0.000	

Values are reported as the Mean ± Standard deviation.

[1]p-values are determined by paired t-test and denote the significance between baseline and final bleaching.

[2]p-values are determined by one-way ANOVA and denote the significance among A1, A2 and A3 groups.

[3]p-values are determined by one-way ANOVA and denote the significance among B1, B2 and B3 groups.

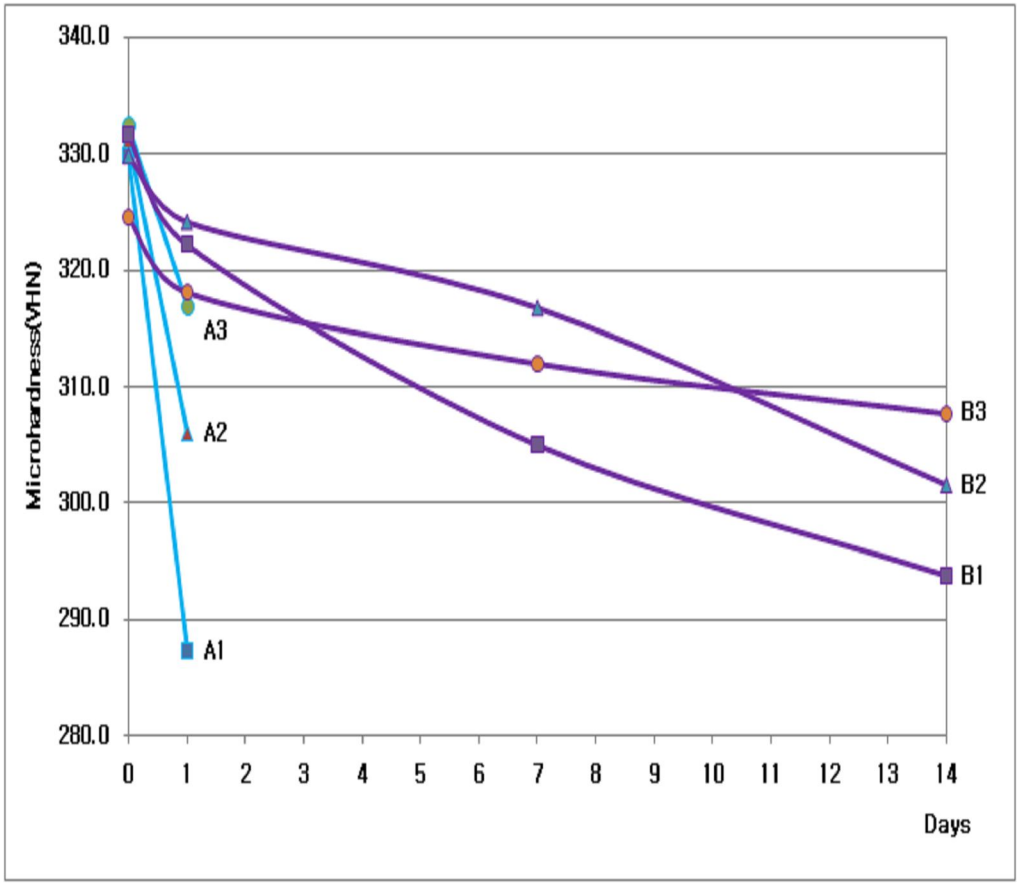

Figure 5. Changes of enamel surface microhardness (VHN) of Group A and B.

Table 10. Mean values of the percentage of microhardness loss (%) according to concentration

Group	PML (%)	p
A1	12.9	0.000
B1	11.4	
A2	7.6	0.000
B2	8.6	
A3	4.6	0.720
B3	5.2	

p-values are determined by t-test.

3.5. SEM analysis

Representative SEM micrographs are shown in Figure 6 and 7. Control groups showed increased surface porosities and erosion-like patterns around the enamel prisms after bleaching. Under higher magnification, the nanocrystals in rods and interrods became distinguishable from each other. Distinct variations of morphology were observed on the enamel surfaces of the groups treated with DCPD / HP compared to the control group.

The surface morphologic changes in Group A2, A3, B2, and B3 were distinct than those of the Group A1 and B1. Prism cores were eroded, surface fracture, cross-striation was apparent, and striae of Retzius were observed. No special alteration was found on the enamel surface in Group A3 compare with those previous study of natural enamel surface treated. A smooth, flat and polished surface was observed.

Enamel surface morphology changes were more prominent in Group A than Group B. With increasing DCPD concentrations, defective enamel surfaces became filled with a crystal. The substantial finding in this study was that DCPD contained groups did not significantly change the enamel surface morphology.

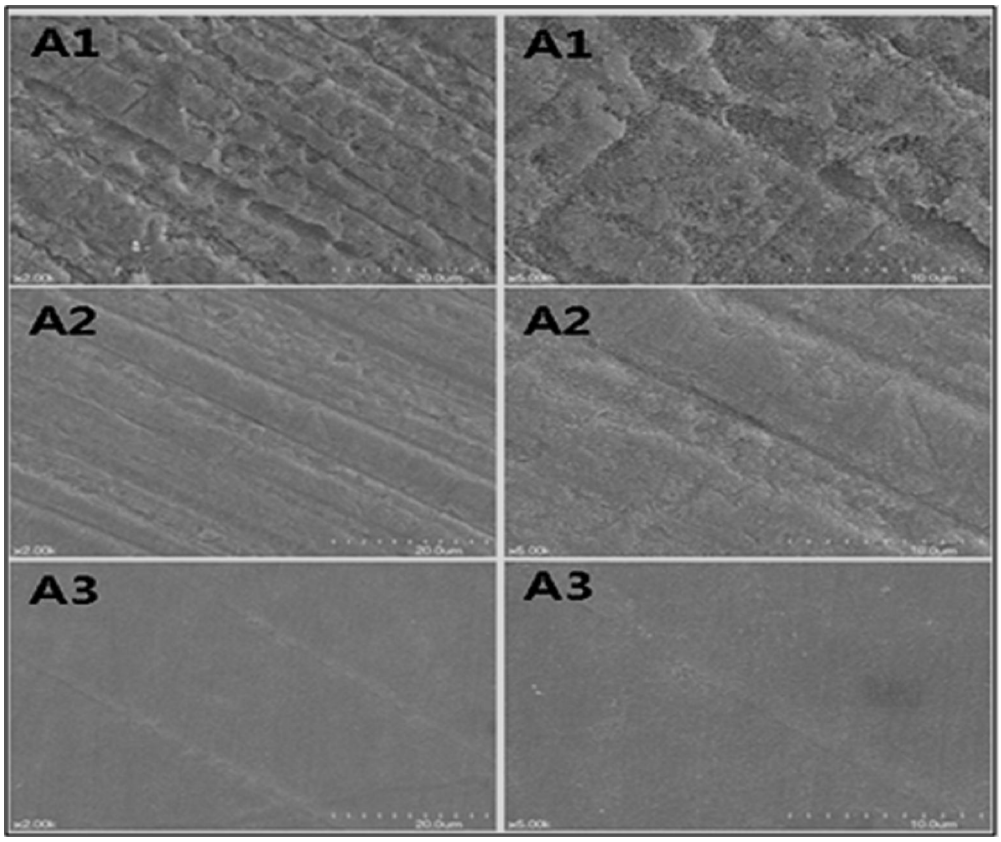

Figure 6. Field emission-SEM photographs of enamel specimens after bleaching with the following groups; (A1) Control, 35 % HP, (A2) 0.1 g DCPD / 35 % HP, (A3) 1 g DCPD / 35 % HP.

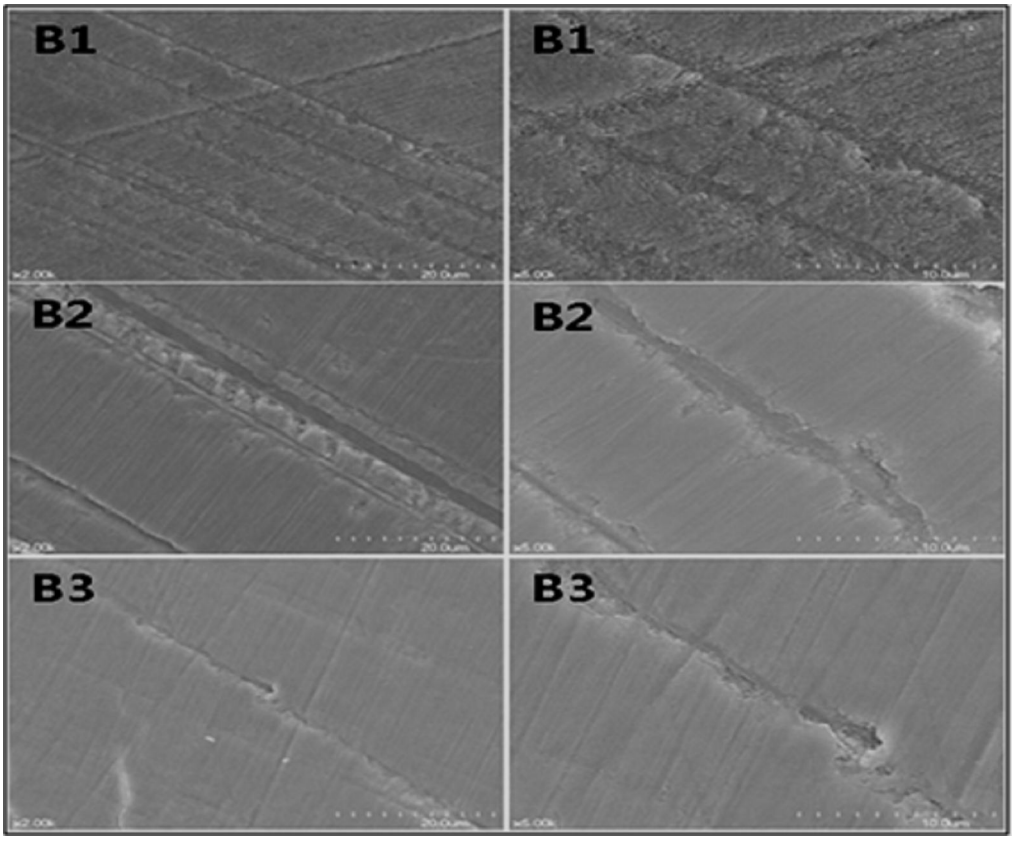

Figure 7. Field emission-SEM photographs of enamel specimens after bleaching with the following groups: (B1) Control, 3.5 % HP, (B2) 0.1 g DCPD / 3.5 % HP, (B3) 1 g DCPD / 3.5 % HP.

3.6. EDX analysis

The experimental data obtained by EDX for all groups after bleaching regarding wt % of Ca, P, and O are displayed in Table 11, Figure 8-9. In DCPD / HP groups, the amount of Ca and P ion found on the surfaces increased with increasing DCPD concentration in the tooth bleaching agent ($p<0.05$). With increasing DCPD concentrations, Ca ions more increased than P ions. The Group A1 showed the lower contents of Ca and P than that of Groups A2, A3, B1, B2 and B3.

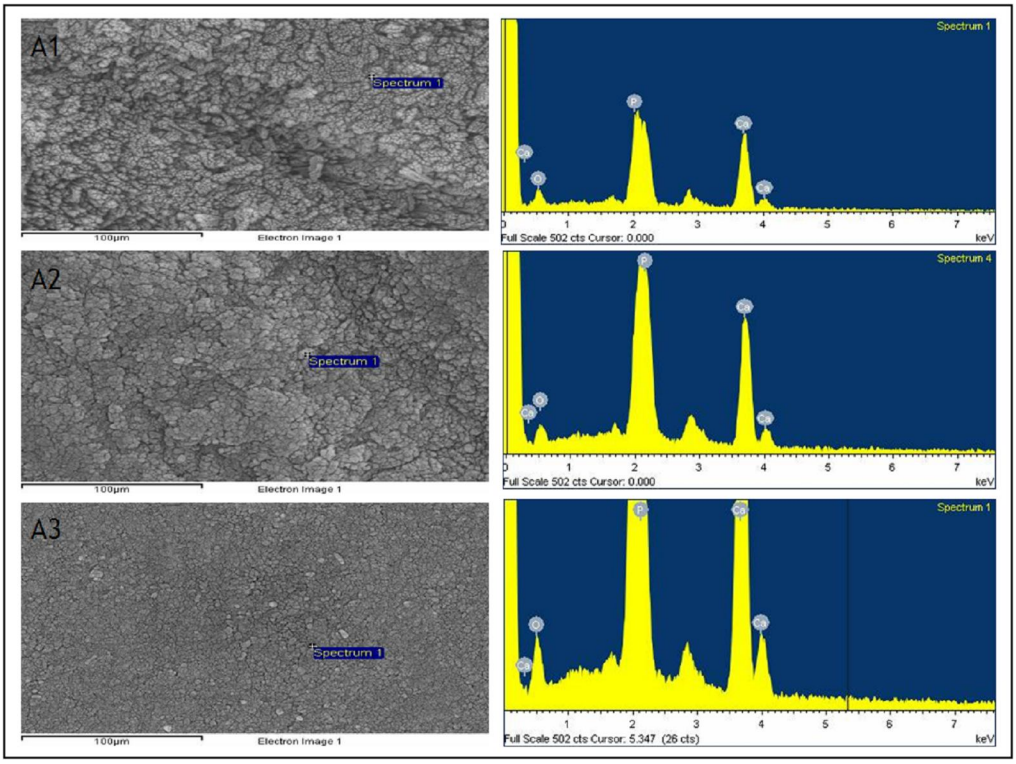

Figure 8. Energy-dispersive X-ray spectroscopy (EDX) photographs of enamel specimens after bleaching of Group A.

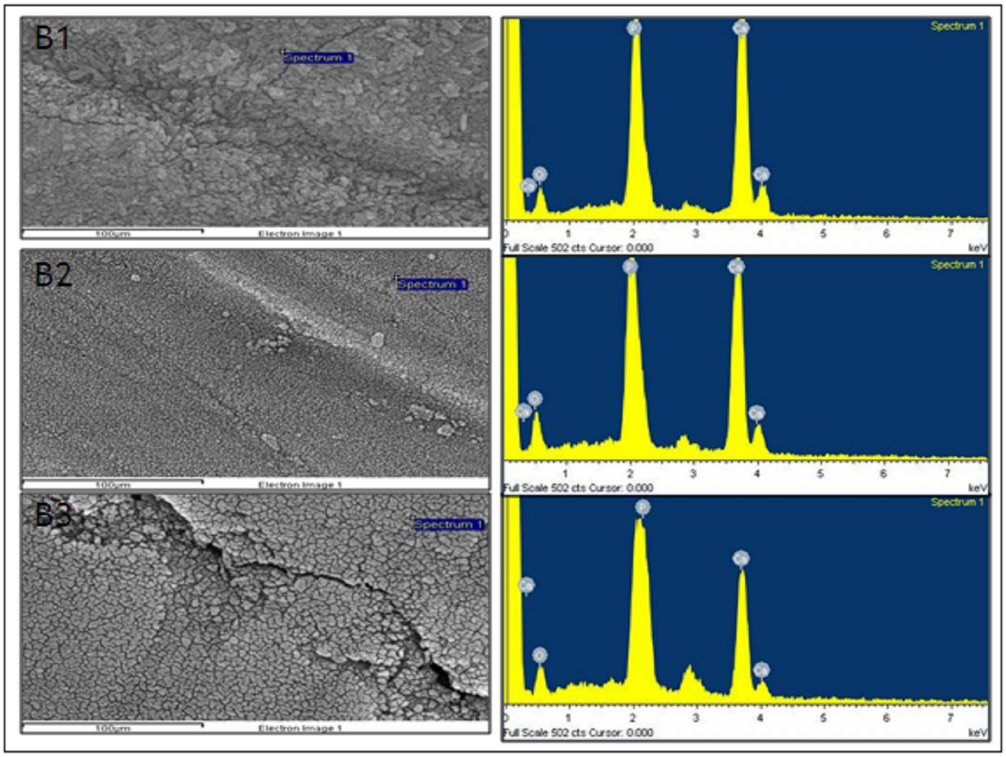

Figure 9. Energy-dispersive X-ray spectroscopy (EDX) photographs of enamel specimens after bleaching of Group B.

Table 11. Contents of calcium, phosphate and oxygen in enamel surface post bleaching

Group	Ca (wt%)	P (wt%)	O (wt%)
A1	40.43 ± 6.2[a]	18.69 ± 1.6[a]	35.76 ± 3.2[d]
A2	50.39 ± 3.9[d]	20.11 ± 1.8[c]	29.49 ± 4.5[b]
A3	53.74 ± 2.4[e]	21.05 ± 1.3[d]	25.21 ± 2.5[a]
B1	43.52 ± 3.7[b]	19.08 ± 3.5[b]	37.40 ± 2.1[e]
B2	46.83 ± 5.3[c]	19.93 ± 3.1[bc]	33.90 ± 3.6[c]
B3	50.21 ± 2.9[d]	20.52 ± 2.1[cd]	29.37 ± 3.2[b]
p[1]	0.001	0.001	0.001

Values are reported as the Mean ± Standard deviation.

[1]p-values were determined by one-way ANOVA ($p<0.05$).

Ⅳ. DISCUSSION

Dental enamel is the most highly mineralized and hardest biological tissue. Enamel is the most dense, the mineral or inorganic phase content amounting to about 95 wt% (87 vol%). It is well known that mature human dental enamel crystals are carbonate-containing HA.[48] DCPD has been known as a precursor phase to HA in the remineralization process. Therefore, DCPD often coexists with HA in the dental hard tissues and interacts with saliva in the oral cavity at the same time.[38]

With increasing DCPD concentration, the pH values in the bleaching agents increased, making it less acidic. This can be explained by the increased supersaturation in the tooth bleaching agent containing DCPD. The low pH of some of the peroxide treatments caused severe changes in enamel topography, particularly the products with very low pH values.[49] Low pH was probably the primary cause of demineralization.[21] The demineralization process of enamel began when the pH fell below 5.5.[50] However, high concentrations of peroxide are also capable of causing enamel surface alterations, despite having close to neutral pH.[21] These results were also confirmed in a previous investigation.

The enamel dissolved at low pH because the calcium and phosphate activities in solution were insufficient to maintain a stable enamel mineral phase.[51] The dissolution well continue until pH and calcium and phosphate activities reach levels where the enamel minerals are stable again. The extent of dissolution depends on a number of factors, such as the pH, the amounts and types of acids produced, and the ionic activities of calcium and phosphate. Note that as the DCPD concentration increased in the bleaching agents, the concentrations of Ca and P ions increased.

Thus, higher pH values may reduce the demineralization effects of acidic HP. In the present study, with increasing pH value, the microhardness values increased, making it less demineralized enamel surface.

The spectophotometric / colorimetric method based on the CIELab system is recognized as a reliable and objective tool for quantitative evaluation of vital tooth color change.[52] The change in color of the enamel specimens in the all groups showed an increase in white (L^*), a decrease in red (a^*) and yellow (b^*). The directional change of these parameters confirm that tooth whitening has occurs. These results are in agreement with other studies, which found the L^* and b^* pre-treatment colorimental results were shown to be affected by bleaching procedures.[53-55] However, these studies did not find any significant difference in the a^* values. The fact that the a^* values after bleaching and one month later went from positive to minus values means that the redness changed to greenness, which could be due to the fact that tea was used to discolor the teeth.[56] The reduction in redness (a^*) represents, in a minor extent, a color improvement, once that the reduction in b^* occurs more rapidly and to a great extent, agreed with the results of the current study.[57] Additionally, the fact that the changes that occurred at the a^* values of Group A were vary small compared with the changes to the Group B values could be the reason for the different results of the studies performed under different protocols. After the conversion of the mean △E values of all specimens to NBS units, it was determined that clinically detectable color changes occurred for all groups.

Several authors have shown that color differences greater than 1△E unit are visually detectable by 50 % of human observers, although △E values less than 3.3 are considered clinically insignificant.[58] Most Importantly, the data indicate

that the addition of DCPD did not influence the whitening efficacy of the bleaching agent negatively.

This study showed that the whitening effect of DCPD / HP agents were similar to that of HP alone. Group A bleaching agents were clearly more efficient than Group B agents, based on the times of application.

The mean Vikers microhardness values for enamel before and after bleaching for the all groups showed a tendency to decrease the microhardness. However, the DCPD concentration increased in the bleaching, microhardness values less decreased (Group A3, B3). in contrast to the study by Polydorou and others, where bleaching with different concentration of bleaching agent resulted in no significant difference enamel microhardness values.[41-43] Other studies have reported significant changes in surface microhardness after bleaching. In the results of the current study, which is in agreement with Pinto and others, who reported that bleaching could significantly decrease enamel microhardness during in vitro treatments.[22,24] The variability of hardness loss in different studies was contributed to factors including the test methods, the immersion media and exposure time.

The initial mean Vikers microhardness values for the six groups ranged from 324 to 332 VHN for the enamel. The decreased microhardness in all group after bleaching were 5 ~ 12 % reduction in microhardness. Previous investigations reported 12 ~ 40 % microhardness losses on 10 % CP treated enamel using a hardness test.[23] The degree of demineralization change caused by the bleaching agents was related to their concentration and the pH value. In regard to the results about microhardness, the decreasing degree of microhardness was significantly greater in the group where a high-dose tooth bleaching agents were included as compared with the group B where a low-dose tooth bleaching agents

were included. 10 % CP is equivalent to about 3.5 % HP. Because the HP content of 3.5 % HP is almost 10 times lower than 35 % HP. Although, therefore, a decrease in microhardness is associated with bleaching no definite relationship has been established here between the percentage decrease in microhardness with the percentage increase in HP concentration of the bleaching agent.

In this study, we found that the group DCPD / HP was still slight reduction in enamel microhardness. However, the oxidation of enamel protein may also result in change of the mechanical properties. A recent study proved that not only the carbamide peroxide, but also other ingredients of bleaching gels, such as the thickening agent carbopol and glycerine may contribute to microhardness loss of bleached enamel.[59]

However, the Group A3 that had 1 g DCPD in the agents showed less significantly loss of hardness than the control group. DCPD seemed to enhance the microhardness of the enamel surface because the samples in that group were piled up with calcium and phosphate. Previously reported work to investigate the demineralization effects of bleaching agents on tooth structures has primarily used microhardness technique. Traditionally, microhardness changes are related to a loss or gain of mineral (demineralization or remineralization) of the dental structure.[60] An increase of microhardness was observed in the fourth week due to exposure to carbamide peroxide could be moderated or controlled through concomitant exposure to saliva.[61]

Scanning electron microscopy (SEM) is a rapid and convenient method for qualitatively analysing the surface morphology of enamel and dentin specimens following bleaching.[62] Control groups showed increased surface porosities and erosion-like patterns around the enamel prisms after bleaching. As in the current

study, Several other studies have reported morphological changes including pitting, waviness and increased surface roughness. Tao et al. observed that the 30 % HP solution resulted obvious variation of enamel surface morphology.[22] Oltu and Gurgan, in 2000, showed structural alterations after the use of 35 % CP.[63] Titley et al, in 1988, demonstrated that after exposure to 35 % HP for 1, 3, 5, 10, 20 and 60 min, structural enamel alterations became more severe.[64] Türkun et al, in 2002, observed through SEM an increase in surface porosity and erosive surface defects, immediately after bleaching procedure.[65] These results support the current findings. In contrast, Gultz et al., in 1999, found no structural alterations in enamel exposed to 35 % CP for 2h or 35 % HP for 20 min.[43]

The observations underline the findings mentioned above, that HP in combination with DCPD seems to affect the enamel surface more less than control group. The specimens treated with DCPD bleaching agents in this study showed only minor erosive patterns and crystal deposition on enamel surfaces.

It was suggested that morphological change of enamel is due to the demineralization caused by acidic HP, while microhardness loss to the combined effects of demineralization and destruction of organic matter by HP. Since the period for rehardening of enamel after application of bleaching agents seems to be extensively long, this might result in an increased susceptibility to surface abrasion.[23] These erosive lesions are susceptible a consequence of acid attacks and abrasion such as tooth brushing in vivo which may lead to irreversible surface loss.[66] However these alterations were reversed within 3 months following treatment.[65]

DCPD dentifrices increase the activities of Ca and P in the plaque fluid, which might increase the driving force for remineralization. The Ca activities remained elevated even 12 hrs after the last treatment.[67] Studies in vitro have

shown that some treatment, including the amorphous calcium phosphate (ACP), can be shown to produce deposite on the dentin surface and or within the tubules or reduce dentin permeability.

The hydrogen peroxide can promote chemical alterations in the composition of the tooth, reducing in quantity of calcium and phosphate in enamel and dentin.[68,69] Studies of tooth mineral release due to exposure to extrinsic agents including HP and acidic carbonated beverages have been carried out. Calcium and phosphorus are present in the hydroxyapatite crystal, the main building block of dental hard tissues. Changes in the Ca / P ratio indicate alterations in the inorganic components of HA.[67]

The EDX analysis showed that the release of Ca and P ions increased with increasing DCPD concentrations. In addition, the release of C and P ions increased with increasing hydrogen peroxide concentrations. Mineral loss values obtained in this study are in correlation with Tao et al's study.[22] The release of Ca ions was consistently greater than release of phosphorous ions at all HP concentrations, for both enamel and dentin.[14] Potocnik and Gaspersic[70] using electron probe microanalysis showed lowered concentrations of Ca and P and with mean Ca / P value of all bleached samples decreasing after bleaching with 10 % CP. Lee et al.[71] investigated mineral loss from bovine enamel by the 30 % HP solution. They reported a decrease in Ca / P ratio of bleached enamel. The liner relationship between the decrease in enamel hardness and Ca and P loss shows that hardness measurements can be used as an indication of the degree of enamel mineralization, which relates to enamel caries.[72] Therefore, the remineralization supplement either during or after bleaching is considered beneficial for preventing enamel demineralization.

The demineralization process begins with the loss of mineral from the surface apatite crystals. Under normal circumstances, this loss of calcium (demineralization) is compensated by the uptakes of calcium (remineralization) from the tooth's microenvironment. This dynamic process of demineralization and remineralization takes place more and less continually and equally in a favorable oral environment. In an unfavorable environment, the remineralization rate does not sufficiently neutralize the rate of demineralization, and thus caries occurs. Also, during decalcification calcium ions have preferentially been removed from areas under the surface, creating an artificial lesion. Demineralization, or loss of mineral content from the outer tooth structure, alters enamel microhardness.[72]

Huseyin et al.[74] found that 35 % HP with light and 38 % HP, may cause significantly more loss of Ca^{2+} from the enamel surfaces than 10 % CP. 10 % CP does not vary significantly from the control. This result corresponds the current study. Calcium phosphate is the main inorganic component of the tooth, calcium phosphate minerals are ideal candidates for obstructing dentin tubules and reducing sensitivity. High concentrations of Ca and P were used to increase the diffusion rates of the ions and, hence, the remineralization rates.[46] Bleaching agents containing of 1 g DCPD were more assessible for ion diffusion and, therefore, should be more susceptible to rapid remineralization.

Other factors that may contribute to the discrepancy in results among previous studies may be both the duration or storage medium of the post bleaching period before testing. Some in vitro studies used artificial saliva or fluoride products between of after the treatments, for these elements are known to be an important factor to simulate clinical situations. However, the aim of this study was to investigate the protective effects of DCPD on the enamel surface subjected to HP. This study did not involve these elements in order to prevent

the influences of any other remineralization factors except DCPD. Nevertheless, it is necessary to involve these factors in the future studies to investigate the beneficial effects of DCPD under typical clinical conditions.

In the current study, regarding the hypothesis about tooth whitening, a null hypothesis was accepted because there was no significant difference in the tooth whitening between tooth bleaching agents with DCPD and those without it. Besides, regarding the hypothesis about enamel surface properties, there was a positive effect in tooth bleaching agents with DCPD. Accordingly, a null hypothesis was rejected.

In this study, the experimental results suggest that DCPD / HP agent less demineralization changes such as the erosion morphology and hardness loss without compromising whitening efficiency.

Ⅴ. CONCLUSION

The present study examined the changes in pH, color, microhardness, and surface morphology and mineral composition of enamel surface treated with different bleaching agents that either contained or did not contain DCPD.

1. The tooth bleaching agents with DCPD showed a significant increase in pH as compared with the ones without DCPD in the group A ($p<0.05$). In the group B, however, there was no statistically significant difference ($p>0.05$).

2. As the concentration of DCPD was increased, the concentration of Ca and P was also increased. The ratio of Ca/P was found to be approximately 1.1 ~ 1.2.

3. In all groups, after the tooth whitening, the tooth color was found to have a value of L^* which was significantly increased, while that of a^* and b^* were significantly decreased as compared with the ones that were seen prior to the tooth whitening ($p<0.05$).

4. In all groups, the hardness of tooth after bleaching showed a significant decrease in the microhardness as compared with the one prior to tooth bleaching ($p<0.05$). In both group A and B, however, as DCPD content was increased, the decreasing degree of microhardness was found to be smaller in the experimental group as compared with the control group.

5. Following an analysis of the characteristics of enamel surface after bleaching, there were porosity and erosion in the control group. In the groups where the amount of DCPD was the greatest, however, there was no surface change.

6. Following an analysis of the constituents of enamel surface after bleaching, as DCPD content was increased, the amount of Ca and P was significantly increased ($p<0.05$).

Based on the above results, tooth bleaching agents of 35 % and 3.5 % of HP containing DCPD are equally effective to the control group. By raising the pH and thereby effectively reducing the decalcification of tooth surface, a lower degree of the effects are given to the surface characteristics and constituent alterations of enamel. Thus, the commercial availability can be achieved for the constituents of tooth whitening materials.

REFERENCES

1. Kihn PW. Vital tooth whitening. Dent Clin North Am 2007 Apr;51(2):319-331.

2. Summitt JB, Robbins JW, Schwartz RS. Fundamentals of operative dentistry : A Contemporary Approach, 3rd. Edition. Quintessence Publishing Co, Inc 2006:437-461.

3. Sulieman MA. An overview of tooth-bleaching techniques: chemistry, safety and efficacy. Periodontol 2008;48:148-169.

4. Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent J 2001 Mar 24;190(6):309-316.

5. Jordan RE, Boksman L. Conservative vital bleaching treatment of discolored dentition. Compend Contin Educ Dent 1984 Nov-Dec;5(10):803-5, 807.

6. Haywood VB, Heymann HO. Nightguard vital bleaching. Quintessence Int 1989;20:173-176.

7. Dahl JE, Pallesen U. Tooth bleaching--a critical review of the biological aspects. Crit Rev Oral Biol Med 2003;14(4):292-304.

8. Kashima-Tanaka M, Tsujimoto Y, Kawamoto K, Senda N, Ito K, Yamazaki M. Generation of free radicals and/or active oxygen by light or laser irradiation of hydrogen peroxide or sodium hypochlorite. J Endod 2003 Feb;29(2):141-143.

9. Theodore M. Roberson, Art & science of operative dentistry, fourth edition 2002.

10. Goldstein RE. Bleaching teeth: new materials--new role. J Am Dent Assoc 1987 Dec;Spec No:44E-52E.

11. Meireles SS, Heckmann SS, Leida FL, dos Santos Ida S, Della Bona A, Demarco FF. Efficacy and safety of 10% and 16% carbamide peroxide tooth-whitening gels: a randomized clinical trial. Oper Dent 2008 Nov-Dec;33(6):606-612.

12. Kugel G, Kastali S. Nontray whitening. Compend Contin Educ Dent 2000 Jun;21(6):524-526, 528.

13. Brantley DH, Barnes KP, Haywood VB. Bleaching primary teeth with 10% carbamide peroxide.Pediatr Dent 2001 Nov-Dec;23(6):514-516.

14. Leonard RH Jr, Van Haywood B, Caplan DJ, Tart ND. Nightguard vital bleaching of tetracycline-stained teeth: 90 months post treatment. J Esthet Restor Dent 2003;15(3):142-152.

15. Leonard RH Jr. Long-term treatment results with nightguard vital bleaching. Compend Contin Educ Dent 2003 Apr;24(4A):364-374.

16. Rotstein I, Friedman S, Mor C, Katznelson J,Sommer M, Bab I. Histological characterization of bleaching-induced external root resorption in dogs. Endod 1991 Sep;17(9):436-441.

17. Schulte JR, Morrissette DB, Gasior EJ, Czajewski MV. The effects of bleaching application time on the dental pulp. J Am Dent Assoc 1994 Oct;125(10):1330-1335.

18. Thitinanthapan W, Satamanont P, Vongsavan N. In vitro penetration of the pulp chamber by three brands of carbamide peroxide. J Esthet Dent 1999;11(5):259-264.

19. Cohen SC. Human pulpal response to bleaching procedures on vital teeth. J Endod 1979 May;5(5):134-138.

20. McGuckin RS, Babin JF. Alterations in human enamel surface morphology following vital bleaching. J Prosthet Dent 1992 Nov;68(5):754-760.

21. Efeoglu N, Wood D, Efeoglu C. Microcomputerised tomography evaluation of 10% carbamide peroxide applied to enamel. J Dent 2005 Aug;33(7):561-567. Epub 2005 Feb 12.

22. Jiang T, Ma X, Wang Z, Tong H, Hu J, Wang Y. Beneficial effects of hydroxyapatite on enamel subjected to 30% hydrogen peroxide. J Dent 2008 Nov;36(11):907-914. Epub 2008 Sep 2.

23. Zantner C, Beheim-Schwarzbach N, Neumann K, Kielbassa AM. Surface microhardness of enamel after different home bleaching procedures. Dent Mater 2007 Feb;23(2):243-250.

24. Pinto CF, Oliveira R, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. Braz Oral Res 2004 Oct-Dec;18(4):306-311.

25. Barghi N, Godwin JM. Reducing the adverse effect of bleaching on composite-enamel bond. J Esthet Dent 1994;6(4):157-161.

26. Nathoo SA, Chmielewski MB, Kirkup RE. Effects of Colgate Platinum Professional Tooth whitening System on microhardness of enamel, dentin, and composite resins. Compend Suppl 1994;(17):S627-630.

27. Li Y. Biological properties of peroxide-containing tooth whiteners. Food Chem Toxicol 1996;34:887-904.

28. Dahl JE, Becher R. Acute toxicity of carbamide peroxide and a commercially available tooth-bleaching agent in rats. Dahl JE, Becher R. J Dent Res 1995 Feb;74(2):710-714.

29. Shapiro WB, Kaslick RS, Chasens AI, Eisenberg R. The influence of urea peroxide gel on plaque, calculus and chronic gingival inflammation. J Periodontol 1973 Oct;44(10):636-639.

30. Fayle SA, Pollard MA. Congenital erythropoietic porphyria--oral manifestations and dental treatment in childhood: a case report. Quintessence Int 1994 Aug;25(8):551-554.

31. Li Y. Peroxide--containing tooth whiteners: an update on safety. Compend Contin Edue Dent 2000;28:S4-S9.

32. Croll TP. Enamel microabrasion for removal of superficial discoloration. J Esthet Dent 1989 Jan;1(1):14-20

33. Goldstein RE, Garber DA. Complete Dental Bleaching. Chicago: Quintessence 1995

34. Robinson FG, Haywood VB. Bleaching and temporomandibular disorder using a half tray design: a clinical report. J Prosthet Dent 2000 May;83(5):501-503.

35. Beevers. CA. The crystal structure of dicalcium phosphate dihydrate, $CaHPO_4.2H_2O$. Acta Cryst 1958;11:273-277.

36. Brown WE, Patel PR, Chow LC. Formation of Ca HPO4-2H2O from enamel mineral and its relationship to caries mechanism. J Dent Res 1975 May-Jun;54(3):475-481.

37. Tanizawa Y. Reaction characteristics of a tooth-bleaching agent containing H2O2 and NaF: in vitro study of crystal structure change in treated hydroxyapatite and chemical states of incorporated fluorine. J Cosmet Sci 2005 Mar-Apr;56(2):121-134.

38. Stralfors A. The effect of calcium phospate on dental caries in school chidren. J Dent Res 1964 43(6):87-93.

39. Finn SB, Jamison HC. The effect of a dicalcium phosphate chewing gum on caries incidence in children: 30-month results. J Am Dent Assoc 1967 Apr;74(5):987-995.

40. Zhang YP, Din CS, Miller S, Nathoo SA, Gaffar A. Intra-oral remineralization of enamel with a MFP/DCPD and MFP/silica dentifrice using surface microhardness. J Clin Dent 1995;6(2):148-153.

41. Polydorou O, Hellwig E, Hahn P. The efficacy of three different In-office bleaching systems and their effect on enamel microhardness. Oper Dent 2008 Sep-Oct;33(5):579-586.

42. Duschner H, Göz H, White DJ, Kozak KM, Zoladz JR. Effects of hydrogen peroxide bleaching strips on tooth surface color, surface microhardness, surface and subsurface ultrastructure, and microchemical (Raman spectroscopic) composition. J Clin Dent 2006;17(3):72-78.

43. Gultz J, Kaim J, Scherer W, Gupta H. Two in-office bleaching systems: a scanning electron microscope study. Compend Contin Educ Dent 1999 Oct;20(10):965-968, 970; quiz 972.

44. Attin T, Betke H, Schippan F, Wiegand A. Potential of fluoridated carbamide peroxide gels to support post-bleaching enamel re-hardening. J Dent 2007 Sep;35(9):755-759.

45. Baratieri LN, Ritter AV, Monterio S, Andrada MAC, Vieira LCC. Non-vital tooth bleaching: guidelines for the clinician. Quintessence Int 1995;26:597-608.

46. Tung MS, Bowen HJ, Derkson GD, Pashley DH. Effects of calcium phosphate solutions on dentin permeability. J Endod 1993 Aug;19(8):383-387.

47. Sulieman M, Addy M, Macdonald E, Rees JS. The bleaching depth of a 35% hydrogen peroxide based in-office product: a study in vitro. J Dent 2005 Jan;33(1):33-40.

48. Sydney-Zax M, Mayer I, Deutsch D. Carbonate content in developing human and bovine enamel. J Dent Res 1991 May;70(5):913-916.

49. Joiner A. Review of the effects of peroxide on enamel and dentine properties. J Dent 2007 Dec;35(12):889-896. Epub 2007 Oct 26.

50. Feagin F, Sierra O, Thiradilok S, Jeansonne B. Effects of fluoride in remineralized human surface enamel on dissolution resistance. J Dent Res 1980 Jun;59(6):1016-1021.

51. Sullivan RJ, Masters J, Cantore R, Roberson A, Petrou I, Stranick M, Goldman H,Guggenheim B, Gaffar A. Development of an enhanced anticaries efficacy dual component dentifrice containing sodium fluoride and dicalcium phosphate dihydrate. Am J Dent 2001 May;14 Spec No:3A-11A.

52. Joiner A, Dietschi D, Rossier S, Krejci I. In vitro colorimetric evaluation of the efficacy of various bleaching methods and products. Quintessence Int 2006 Jul-Aug;37(7):515-526.

53. Luk K, Tam L, Hubert M. Effect of light energy on peroxide tooth bleaching. J Am Dent Assoc 2004 Feb;135(2):194-201,

54. Rosenstiel SF, Gegauff AG, Johnston WM. Duration of tooth color change after bleaching. J Am Dent Assoc 1991 Apr;122(4):54-59.

55. Gegauff AG, Rosenstiel SF, Langhout KJ, Johnston WM. Evaluating tooth color change from carbamide peroxide gel. J Am Dent Assoc 1993 Jun;124(6):65-72.

56. Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, Pimenta LA. Microhardness evaluation of in situ vital bleaching on human dental enamel using a novel study design. Dent Mater 2005 Nov;21(11):1059-1067

57. Luo W, Westland S, Brunton P, Ellwood R, Pretty IA, Mohan N. Comparison of the ability of different colour indices to assess changes in tooth whiteness. J Dent 2007 Feb;35(2):109-116.

58. Ruyter IE. Nilner K, Moller B. Color stability of dental composite resin materials for crown and bridge veneers. Dent Mater 1987;3:246-251.

59. Basting RT, Rodrigues AL Jr, Serra MC. The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness. Oper Dent 2005 Sep-Oct;30(5):608-616.

60. Featherstone JD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983;17(5):385-391

61. Maia E, Baratieri LN, Caldeira de Andrada MA, Monteiro S Jr, Vieira LC. The influence of two home-applied bleaching agents on enamel microhardness: an in situ study. J Dent 2008 Jan;36(1):2-7. Epub 2007 Nov 26.

62. Joiner A, Thakker G. In vitro evaluation of a novel 6% hydrogen peroxide tooth whitening product. J Dent 2004;32 Suppl 1:19-25.

63. Oltu U, Gügan S. Effects of three concentrations of carbamide peroxide on the structure of enamel. J Oral Rehabil 2000 Apr;27(4):332-340.

64. Titley K, Torneck CD, Smith D. The effect of concentrated hydrogen peroxide solutions on the surface morphology of human tooth enamel. J Endod 1988 Feb;14(2):69-74

65. Tükun M, Sevgican F, Pehlivan Y, Aktener BO. Effects of 10% carbamide peroxide on the enamel surface morphology: a scanning electron microscopy study. J Esthet Restor Dent 2002;14(4):238-244.

66. Vieira A, Lugtenborg M, Ruben JL, Huysmans MC. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. Caries Res 2006;40(3):224-230.

67. Sullivan RJ, Charig A, Blake-Haskins J, Zhang YP, Miller SM, Strannick M, Gaffar A, Margolis HC. In vivo detection of calcium from dicalcium phosphate dihydrate dentifrices in demineralized human enamel and plaque. Adv Dent Res 1997 Nov;11(4):380-387.

68. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. J Endod 1996 Jan;22(1):23-25.

69. Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. J Endod 2004 Jan;30(1):45-50.

70. Potocnik I, Kosec L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content.J Endod 2000 Apr;26(4):203-206.

71. Lee KH, Kim HI, Kim KH, Kwon YH. Mineral loss from bovine enamel by a 30% hydrogen peroxide solution. J Oral Rehabil 2006 Mar;33(3):229-233.

72. Feagin F, Koulourides T, Pigman W. The characterization of enamel surface demineralization, remineralization, and associated hardness changes in human and bovine material. Arch Oral Biol 1969 Dec;14(12):1407-1417

73. Shannon H, Spencer P, Gross K, Tira D. Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. Quintessence Int 1993 Jan;24(1): 39-44.

74. Tezel H, Ertaş S, Ozata F, Dalgar H, Korkut ZO. Effect of bleaching agents on calcium loss from the enamel surface. Quintessence Int 2007 Apr;38(4): 339-347.

ABSTRACT (IN KOREAN)

Dicalcium phosphate dihydrate를 함유한 치아미백제가 치아 미백효과와 법랑질의 표면 특성에 미치는 영향

<지도교수 김 광 만>

연세대학교 대학원 치의학과

심 연 수

최근 심미적 욕구의 증가로 인해 외모에 대한 관심이 증가하고 있으며, 이로써 시술이 용이하고 효과적이며 치질 삭제가 없는 치아 미백에 대한 관심이 증가하고 있다. 치아미백이란 과산화수소가 강력한 산화제로서 활성 산소 라디칼과 perhydroxyl 라디칼로 분해되어 반응한다. 지금까지 많은 연구에서 치아미백제가 법랑질의 표면 특성에 미치는 영향에 대해 아직 의견이 분분한 실정이다.

이 연구의 목적은 Dicalcium phosphate dihydrate를 함유한 치아미백제가 치아 미백과 표면 특성에 미치는 영향에 대해 알아보고자 한다. 제2인산칼슘(Dicalcium phosphate dihydrate, DCPD, $CaHPO_4 \cdot 2H_2O$)은 구강 내 칼슘의 농도를 증가시켜 치면의 재광화를 증가시키는 효과와 치아우식 예방효과가가 있고, 다른 인산칼슘 화합물과 달리 산성용액에서 열역학적으로 안정된 물질이다.

이에 본 연구에서는 35 % Hydrogen peroxide (HP) 와 3.5 % HP를 선택하였고, 60개의 치아 시편을 2군 (A군, B군)으로 나눈 후 (n=30) 각 각을 3개의 subgroup (n=10)으로 나눴다. 35 % HP와 3.5 % HP에 DCPD를 0 g (대조군), 0.1 g, 1 g 함유하여 치아 미백제를 제조했다. A 군 (DCPD / 35 % HP)은 하루에 1시간 치아 미백하였고, B 군 (DCPD / 3.5 % HP)은 하루에 8시간 미백하여

14일 동안 반복하였다. 미백제의 pH 측정, ICP를 이용한 원소분석을 실시하고, 법랑질의 색, 경도, 표면의 형태 및 무기질 성분 분석을 하여 DCPD를 함유한 치아미백제의 미백효과와 법랑질의 표면 특성에 대해 연구하여 다음과 같은 결과를 얻었다.

1. 치아미백제의 pH를 측정한 결과 DCPD를 함유한 치아미백제의 pH는 함유하지 않은 치아미백제의 pH에 비해 A군은 유의한 증가를 보였으나 ($p<0.05$), B군은 통계적으로 유의한 차이는 없었다($p>0.05$).

2. 치아미백제의 무기질 함량을 측정한 결과 DCPD 농도가 증가할수록 칼슘과 인의 농도가 증가했으며, Ca/P 비가 1.1 ~ 1.2 정도를 나타냈다.

3. 모든 군에서 미백 후 치아 색은 미백 전 치아 색에 비해 통계적으로 유의하게 L^* 값은 증가했고, a^* 값과 b^* 값은 감소했다 ($p<0.05$).

4. 모든 군에서 미백 후 치아의 경도는 미백 전 치아의 경도에 비해 통계적으로 유의한 미세경도의 감소를 보였다 ($p<0.05$). 그러나 A군과 B군 모두에서 DCPD 함유량이 증가할수록 대조군에 비해 실험군의 미세경도 감소량이 적게 나타났다 ($A3<A2<A1$, $B3\leq B2<B1$).

5. 미백 후의 법랑질 표면의 형태를 분석한 결과 대조군에서는 표면의 다공성과 침식 현상이 보였으나 (A1, B1), DCPD 함유량이 가장 많은 군(A3, B3)에서는 표면 변화가 나타나지 않았다.

6. 미백 후 법랑질 표면의 성분을 분석한 결과 DCPD 함유량이 증가할수록 Ca, P 함량이 통계적으로 유의하게 증가하였다 ($p<0.05$).

이상의 결과로부터 DCPD를 함유한 35 %와 3.5 % HP의 치아미백제는 대조군과 동등한 치아미백 효과가 있고, pH를 상승시켜서 치아 표면의 탈회를 효과적으로 감소시켜 법랑질의 표면 형태와 성분 변화에 덜 영향을 줌으로써, 치아미백제의 구성성분으로 실용할 수 있을 것으로 사료된다.

핵심되는 말 : 치아미백, DCPD, pH, 색, 미세경도