

Use of a Calcium Peroxide Whitening Agent for Remineralization and Recalcification of Incipient Lesions

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Abstract

- **Objective:** This *in vitro* study examined the ability of Forté, based upon co-catalyzed calcium peroxide and carbamide peroxide (CaCP), to remineralize and recalcify pre-existing incipient lesions in tooth enamel, renewing the teeth while they are being whitened.
- **Methodology:** Artificial lesions to 70-100 micrometers depth were created, simulating *in vivo* conditions. Calcium concentrations were determined by micro drill, and surface hardness was determined using a Leco Indenter. Remineralization and calcium uptake were determined by comparing the surface hardness and calcium concentration of subsurface incipient lesions in extracted human teeth before and after a whitening treatment, and also comparing to a non-whitening control (saliva).
- **Results:** Specimens treated with the CaCP whitening gels had a significant calcium uptake of 33,000 micrograms of calcium/gram of enamel measured to a depth of 100 micrometers, and had corresponding increases in surface hardness, from a Vickers Hardness Number of 40 VHN to a value of 83 VHN.
- **Conclusion:** Recalcifying incipient lesions to clinically significant depths, and improving the surface hardness of enamel while performing a popular cosmetic bleaching service, compares favorably with losses of calcium and decreased enamel surface hardness, as reported in studies of many conventional whitening agents.

(J Clin Dent 18:00-00, 2007)

Introduction

Teeth bleaching has evolved as an integral part of esthetic dentistry.¹ The American Dental Association (ADA) recognizes teeth bleaching and has accepted bleaching with a 10% carbamide peroxide bleach or equivalent as safe and effective. The ADA's position is consistent with the findings from a study by McCracken and Heywood.² In that study, a 10% carbamide peroxide bleach was applied for six hours. The teeth lost about one microgram of Ca/mm² of exposed enamel, comparable to soaking the teeth in cola for 2.5 minutes, and determined that the loss may be clinically acceptable.

Much has been written on the detrimental effects peroxides have on microhardness, but that the use of remineralizing agents and saliva may repair some or all of the damage done to the enamel.³⁻¹⁰ Calcium losses in the subsurface of enamel up to 15% were found in studies using 10% carbamide peroxide.¹¹ This wide range of study results—from loss of surface hardness and loss of subsurface calcium, to conclusions that tray-delivered, dentist-supervised bleaching is safe and effective—suggest that there still is some controversy surrounding this very popular part of dentistry.

The whitening agent in this study, Forté (Nu Radiance®, Inc., Green Bay, WI, USA¹²), at the time of whitening, blends and co-catalyzes calcium peroxide and carbamide peroxide and sustains this blend (hereinafter CaCP) in a pH range of 9.5–10.5. This high pH scavenges the hydrogen ions from the phosphates¹³ H₃PO₄, H₂PO₄⁻, and HPO₄²⁻, increasing PO₄³⁻ (Figure 1). Such

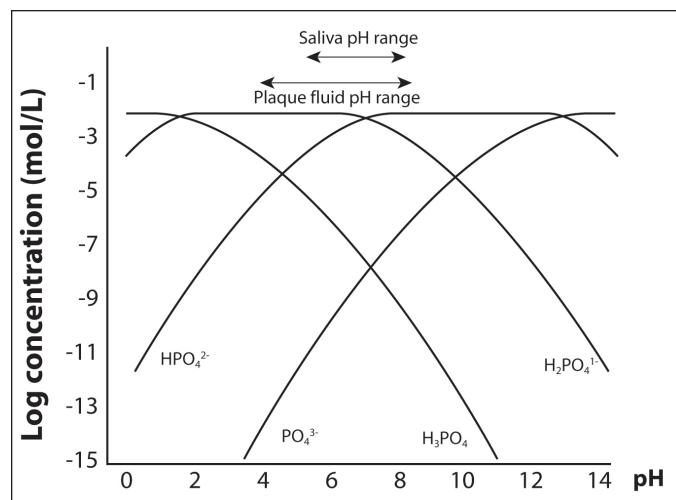


Figure 1. Describes the phosphate ion distribution for a phosphate solution such as saliva at 5×10^{-3} mol/L. The distribution functions apply for any inorganic phosphate solutions.¹³

scavenging assures super-saturation of hydroxyapatite precursors, while the precursors of more acidic calcium phosphates become under-saturated. As the CaCP gels release peroxides, or at times near, the activated calcium gels release tremendous amounts of calcium ions within the voids in the enamel and dentin. This combination of events favors the precipitation of apatite crystals within the lesion and dentin porosities¹⁴⁻¹⁷ during the teeth bleaching process.

Anecdotal information focused on the disappearance of surface defects, the calcification of post-orthodontic white spots, the disappearance of "soft spots" in enamel, and the lack of sensitivity when using Forté as compared to other whiteners. Calcium uptake to the teeth, suggested by the anecdotal information, is consistent with studies done with high pH solutions, and by studies on mass precipitation of $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$.^{15,16}

Previous unpublished laboratory studies by the authors evaluated the CaCP gels qualitatively. These studies, which used one 4.4 ml dual-chambered auto-mix syringe of the CaCP gels, demonstrated calcium uptake when compared to a 16% carbamide peroxide whitener and to glycerin (the control). The study reported here expands this research to a complete whitening treatment, and compares quantitatively remineralization and calcium uptake produced by the CaCP gels to that achieved by saliva alone (with no whitening gel). The purpose of this study was to measure the ability of the CaCP gels to promote remineralization of enamel while bleaching teeth, and to measure its ability to recalcify pre-existing incipient lesions in enamel, regardless of their origin.

Materials and Methods

Preparation of Specimens

Human teeth were collected from oral surgeons in a pooled fashion so there was no chance of identifying donors, and thus eliminating any confidentiality issues. Enamel specimens 3 mm in diameter were removed from these extracted human teeth and mounted in rods. The specimens were ground and polished to a high luster with gamma alumina using standard methods. Twelve specimens per group were prepared for this study. Artificial lesions were formed in the enamel specimens by a 72-hour immersion into 14.0 ml of a solution of 0.1 M lactic acid and 0.2% Carbopol C907, which had been 50% saturated with hydroxyapatite and adjusted to pH 5.0 at 37° C. Upon completion of the lesion formation, the lesion surface hardness range was 25–45 (Vickers Hardness Number),¹⁸ and average lesion depth was approximately 70 micrometers.

Measurement

The hardness was measured with a Vickers Indenter at 200 g load for 15 seconds using a Leco hardness tester (Leco Corp., St. Joseph, MI, USA). Four indentations per specimen were averaged at both pre- and post-test time periods. The baseline calcium content of each specimen was measured prior to treatment using the micro-drill sampling technique to 100 micrometers depth. The removed enamel was then dissolved in 1N HClO_4 and prepared for analysis using standard methods with atomic absorption. The amount of calcium in the analyzed sample was then determined.

Saliva Collection

A mixture of pooled human saliva was used as the remineralization medium for all treatment regimens. Wax-stimulated saliva was collected from at least three healthy individuals and refrigerated until used. Saliva samples were then pooled with vigorous stirring prior to distribution into 50 ml treatment beakers. Fresh saliva was used three times each day (see *Treatment Regimen*).

Testing Materials

Unlabeled dual-chamber treatment syringes containing Forté whitener gel were provided by the manufacturer. Forté is a dual-component whitening gel containing 14% calcium peroxide in one chamber and 30% carbamide peroxide in the second chamber, which are combined at the time of application by the mixing tip on the syringe. (The mixed and activated gels are referred to as CaCP gels.)

The test design consisted of the following treatment solutions:

1—Whitening gel only (15 g)

2—Whitening gel mixed 80:20 with saliva (12 g gel: 3 g saliva) immediately prior to exposure.

3—Saliva only (15 ml)

The 80:20 blend was included in the testing to determine, qualitatively, the interference to recalcification of incipient lesions associated with lower pH, due to premature deposition of acidic calcium phosphates at the surface of the teeth.

Treatment Regimen

During the treatment period, the prepared specimens were immersed in their designated solutions to simulate daily treatment. The gel solutions were prepared by extruding gel from the provided syringes. A fresh gel (15 g) was prepared by proper extrusion just prior to each treatment. The gel (or gel plus saliva) was placed into a treatment beaker and mixed well immediately prior to placing the specimens into the treatment. Treatments were performed at room temperature (24°C), an accepted convention for studies with frequent change-over requirements and with macro-parameters being evaluated.

The daily cyclic treatment regimen consisted of three, 1½-hour treatment periods with the specific treatment solution (Figure 2). After each treatment, the specimens were rinsed with running distilled water then placed back into saliva. The specimens were in the pooled human saliva for the remaining time during the day. In this model, there was no acid challenge period or fluoride treatment. Overnight, the specimens were allowed to stand with a film of saliva on them to simulate sleep. They were not immersed; just removed from the saliva exposure and placed in a sealed container. The regimen was repeated for 10 days.

a.	7:00–8:30 a.m.	Treatment‡
b.	8:30–10:00 a.m.	Saliva alone
c.	10:00 a.m.–11:30 a.m.	Treatment
d.	11:30–1:00 p.m.	Saliva alone
e.	1:00–2:30 p.m.	Treatment
f.	2:30–4:00 p.m.	Saliva alone
g.	4:00 p.m.–7:00 a.m.	Salivary film overnight
h.	Back to (a)	

‡Fresh saliva changed.

Figure 2. Treatment regimen.

Remineralization

Remineralization was determined by comparing the surface hardness of the lesion area after the whitening treatment to the surface hardness of the lesion area before the whitening treatment, and to a control (saliva). The saliva-only experiment was included as the control to assure single parameter integrity.

Calcium Uptake

Calcium uptake was determined by comparing the calcium content within the lesion area after treatment to the calcium content of the lesion area before treatment, and to the calcium content of the lesions maintained in saliva only (control). The calcium content of each specimen following treatment was measured using the micro-drill sampling technique to a depth of 100 micrometers. The removed enamel was then dissolved in 1N HClO₄ and prepared for analysis using standard methods with atomic absorption. The amount of calcium in the analyzed sample was determined and compared to the pre-treatment calcium levels and to the control. While the incipient lesions formed were to a depth of approximately 70 micrometers, the calcium concentration was measured to a depth of 100 micrometers to be sure to include the entire lesion depth. (Such measurements would underestimate the calcium concentration uptake and thus would be conservative.)

Data Analysis

The mean and SEM of each parameter for each group were calculated. Statistical analysis was performed with a one-way analysis of variance model using Sigma Stat (3.1, Systat Software, Inc, San Jose, CA, USA) software. Since the ANOVA test indicated significant differences, the individual means were analyzed by the Student-Newman-Keuls (SNK) test.

Results

Remineralization

Figure 3 and Table I show the surface hardness of the enamel exposed to the action of the CaCP gels, exposed to a blend of the

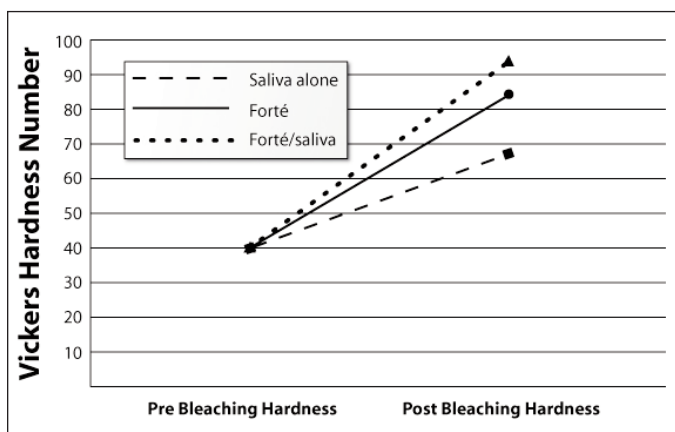


Figure 3. Surface hardness of enamel.

Table I
Summary of Hardness Changes

Treatment Solution	Pre-Test* Hardness	Post-Test Hardness	Change
Saliva Alone	41.0 ± 1.1†	66.7 ± 1.5	25.7 ± 1.4
Gel Alone	40.6 ± 1.3	83.2 ± 4.1	42.6 ± 3.2
Gel Mixed with Saliva	40.9 ± 1.1	92.2 ± 2.9	51.4 ± 2.3

*Values in this column do not differ significantly (p > 0.05) as determined by Student-Newman-Keuls analysis. Values in the other two columns are all different (p = 0.043 or less).

†Mean ± SEM (N = 12).

CaCP gels and saliva, and exposed to saliva alone. The results show that both experiments with the CaCP gels had greater increases in surface hardness than obtained with saliva alone.

Calcium Uptake

The mean of measured increases in calcium uptake into the volume of the lesions of the enamel was approximately 33,000 micrograms Ca/gram of enamel when measured over the 100 micrometer depth for the CaCP gel experiment (Figure 4 and Table II).

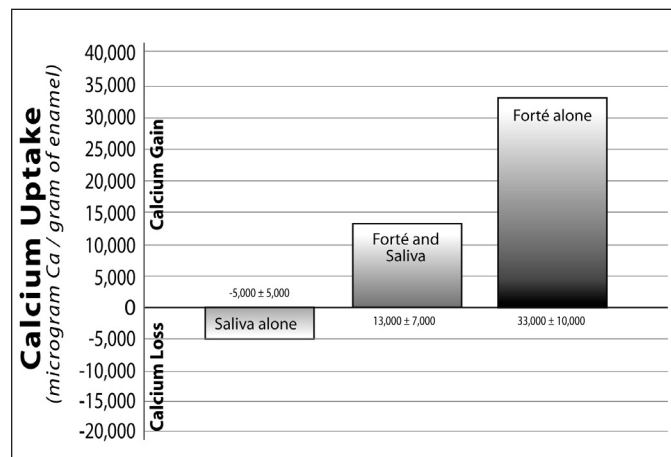


Figure 4. Calcium uptake using Forté.

Table II
Summary of Calcium Changes, microgram Ca/g of Enamel

Treatment Solution	Pre-Test	Post-Test	Change
Saliva Alone	142500 ± 3500*†	138000 ± 2500	-5000 ± 5000
Gel Mixed with Saliva	155000 ± 5000*†	167500 ± 5000	13000 ± 7000
Gel Alone	147000 ± 5000*†	180500 ± 7500	33000 ± 10000

*Mean ± SEM (N = 12).

†Values within brackets do not differ significantly (p > 0.05) as determined by Student-Newman-Keuls analysis.

Discussion

Remineralization of the incipient lesion area, measured by enamel surface hardness increase, was significantly greater with the CaCP gel and with the CaCP gel/saliva mixture than with saliva alone. Additionally, the calcium uptake data indicated that both the gel and the gel/saliva treatments promoted higher calcium uptake into the body of the incipient lesion than did the saliva alone treatment. The teeth exposed to the CaCP gel alone group had a significantly greater calcium uptake into the lesion area than did the teeth that were exposed to saliva alone. Mineralization within the body of incipient lesions, measured by calcium uptake, when using the high pH CaCP gels is consistent with prior studies.^{15-17,19} The previous unpublished data showed the calcium uptake with one syringe was about proportional to the uptake with some five syringes of gel over an extended period of time, suggesting a cumulative effect.

Phosphates in solution, saliva, plaque fluid, or otherwise are made up of the various ionic groups, distributed as functions of the local pH, as is shown in Figure 1. As the pH increases, the phosphates in solution become supersaturated with respect to

apatite, and under-saturated with respect to the more acidic phosphates. In the experiments by Silverstone, *et al.*,¹⁵ apatite crystals were preferentially deposited deep within lesions using a supersaturated apatite solution and under-saturated solutions for the acidic phosphates. In the experiment by Soulayman,¹⁶ apatite crystals were mass-deposited over a 30-minute period, with no interim unstable acidic phosphates due to the high pH (over 9.0). In the experiment by Tung, *et al.*²⁰ with dentin disks, apatite crystals were deposited within the tubules in a two-step application of calcium and phosphates at a high pH of 9.5

The experiments with saliva and saliva blended with CaCP gels indicate possible premature deposition of the calcium phosphates, and blockage from further re-calcification when compared to the results of the experiment with the CaCP gel alone. These findings are consistent with the premature deposition of unstable calcium phosphates on the surface of the teeth.^{15,21}

The increase in calcium concentration and the increase in enamel hardness in the CaCP gel experiment suggest that the increase in calcium is in the form of apatite.¹⁵ The increase in microhardness from VHN 40 to VHN 83 for the CaCP gels surface hardness measurements indicates a mineral uptake, measured by calcium content, of approximately 30,000 micrograms Ca/gram of enamel, based upon the Featherstone, *et al.* correlation.²² The increase in calcium concentration in Figure 4 shows 33,000 micrograms Ca/gram of enamel measured over 100 micrometers, suggesting that the mineral uptake into the lesion is apatite mineral, is consistent with the surface hardness, and is contributing to the structure of the enamel within the lesion.

Impact on Dentin

CaCP gels may also promote deposition of apatitic mineral within the exposed tubules, occluding them and reducing sensitivity. The history of Forté use shows little to no sensitivity being reported. As the calcium phosphates are precipitating at a high pH, the crystals should be small and apatite in nature, consistent with the precipitations within the enamel. The deposits would also take place within exposed dentin tubules and would block fluid flow, reducing, if not eliminating dentinal hypersensitivity consistent with Brannstrom's²³ hydrodynamic theory and previously noted studies of calcium phosphates in a high pH environment.¹⁷

Conclusions

When whitening teeth, the uptake of 33,000 micrograms Ca/gram of enamel in the form of an apatite mineral within incipient lesions, concomitant with the increased enamel microhardness, compares favorably with the loss of calcium and microhardness, even minimal, as identified in various studies when using other whiteners. These *in vitro* studies indicate that the Forté teeth whitener, with its high pH and calcium peroxide blended with carbamide peroxide (CaCP), recalcifies pre-existing incipient lesions to significant depths within enamel, and increases the surface hardness of the enamel during a teeth whitening (bleaching) procedure.

A whitener that promotes significant remineralization, as measured by surface hardness improvement and calcium uptake into incipient lesions, provides the practitioner with a teeth whiten-

ing alternative that improves the health of the teeth. Not only can the use of a calcium peroxide whitener prevent the problems of demineralization, the practitioner can recalcify and remineralize incipient lesions, restoring the health of the enamel while providing a cosmetic service that the patient seeks.

Further study on the possible applications, including dentin, deeper lesions, mineral structure, and sensitivity to the distribution of the reagents are suggested. A study on the calcifying of incipient lesions in a population prone to soft spots would also be appropriate.

Acknowledgment: This study was supported by Nu Radiance, Inc.

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