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Research Article

# Remineralization Potential of Different Agents and Assessment by a New Caries Detection Device

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## Abstract

**Objectives:** The aim of this study was to evaluate the effectiveness of casein and hydroxyapatite on remineralization of white spot enamel lesion and assessment by a new caries detection device, FluoreCam System (Thereametric Co., Indianapolis, USA).

**Study Design:** Demineralized human enamel specimens were measured for baseline surface by the following methods; micro hardness, Quantitative Light Induced Fluorescence (QLF) (Inspektor Pro, Inspektor Research Systems, Amsterdam, Holland) and FluoreCam System (There a metric Co., Indianapolis, USA). Ten specimens in each of four groups were used in this in vitro recycling study with the following treatments applied three times daily for 1 min: 1) Sodium fluoride (NaF) dentifrice, Ipana, (Procter&Gamble, Cincinnati, Ohio, USA), 2) Casein phosphor peptide-amorphous calcium phosphate (CPP-ACP) agent, Tooth Mousse, (Recaldent, GC Europe, Hamburg, Germany), 3) hydroxyapatite and fluoride agent, Remin Pro, (Voco, Cuxhaven, Germany), 4) Fluoride varnish, Pro fluorid, (Voco, Cuxhaven, Germany). The recycling demineralization-remineralization treatment regimens were continued for 21 days. The post-treatment data were obtained by measurements of surface micro hardness, QLF and FluoreCam System (Therametric Co., Indianapolis, USA). Statistical analyses of the data included ANOVA test with Tukey's HSD test.

**Results:** Significant differences between treatments were observed by micro hardness; compared to the positive control group (NaF dentifrice) enhanced and significantly greater remineralization was observed with the Remin Pro treatment ($p \leq 0.001$). However no significant differences between groups were observed using the fluorescence assessments.

**Conclusions:** The application of Remin Pro, Tooth Mousse and Pro fluorid showed remineralization potential on pre white spot enamel lesion under the condition of this study. FluoreCam System seems promising on detection of early lesion and its remineralization.

**Clinical Significance:** Pre white spot enamel lesion can be remineralized by the agents used in this study and can be detected by new QLF system.

## Keywords

FluoreCam; Remin Pro; Remineralization; Tooth Mousse; White Spot Lesion; QLF

## Introduction

Dental caries is a disease continuum, from the earliest loss of ions from apatite crystals through to lesion cavitation [1]. A goal of modern dentistry is to manage non-cavitated caries lesions non-invasively through remineralization in an attempt to prevent disease progression [2]. In recent years topical gels, varnishes, mouthwashes and dentifrices were developed containing nano-sized bio inspired apatite particles in combination with or without proteinaceous additives like casein phosphopeptide or hydroxyapatite for the treatment of non cavitated caries lesions [3].

CPP-ACP is a casein derived peptide, with added calcium and phosphate, which acts as a calcium and phosphate reservoir when incorporated into dental plaque and on the tooth surface. It has been shown that Casein Phosphor Peptides (CPP) have the capacity to stabilize calcium phosphate in solution through binding Amorphous Calcium Phosphate (ACP) to their multiple phosphoserine residues forming small CPP-ACP clusters [4]. However, CPP-ACP does not mimic nano-sized enamel crystallites. Other biomimetic approaches are based on hydroxyapatite nanocrystals resembling the nanostructure of abraded dental enamel crystallites. Different types and pharmaceutical forms of nano hydroxyapatite promote remineralization and repair of demineralized enamel or micro sized tooth surface defects [5].

Non-destructive techniques for evaluation of mineral content enable long-term assessment of effects of the remineralizing agents on enamel. Light fluorescence is a noninvasive technique and offers considerable potential in diagnosis as well as serving as a tool for research. Caries can be detected by quantitative light-induced fluorescence because the fluorescence radiance at the site of caries lesions is decreased and thus appear as dark spots. One of the methods using fluorescence is Quantitative Light-induced Fluorescence system (QLF) has been shown to be sensitive for the detection of early incipient lesions [6-11]. Based on the restricted data available, it appears that QLF is suitable for in vivo monitoring of mineral changes, as well as for caries preventive programs [12]. A new quantitative light-induced fluorescence method is introduced with similar to QLF and with some advancements and called the FluoreCam System. The FluoreCam System is based on an innovative approach to quantifying enamel health called Fluorescence Enamel Imaging, or FEI. The idea behind FEI comes from the chemical and physical properties of tooth enamel. Enamel is both highly mineralized and semi translucent. Because of its mineral composition, enamel will fluoresce when exposed to certain light wavelengths. The semi translucent nature of enamel results in different enamel densities emitting different levels of fluorescence. The instrument excites the surface of a tooth with a high intensity light and sends the resulting fluorescent image and measurements to a computer [13].

It was the aim of this study to evaluate the remineralization potential of casein, hydroxyapatite and fluoride on remineralization of white spot lesion. The second aim was to evaluate the efficiency of the new detection device; FluoreCam System on detection of early demineralization and remineralization.

## Materials and Methods

Enamel Specimens and Preparation of Subsurface Lesions. A total of 40 human enamel specimens were used for this study. Extracted teeth that have been obtained from oral surgeons were used; the teeth were stored in 0.10% thymol solution immediately after extraction and maintained in this solution prior to use. The sound enamel specimens required for this study were 3 mm in diameter and 1.6-2.0 mm in thickness from the enamel surface. These enamel cores were mounted on acrylic rods. Surfaces of specimens were polished by a 600-grit grinding disk and with slurry of 0.05 um gamma alumina polishing gel. Artificial subsurface carious lesions were formed on each enamel specimen by placing the specimens individually for 72 hours at 37°C in 7.0 ml of a demineralizing solution containing 0.1 molar lactic acid and 0.2% Carbopol 907, 50% saturated with hydroxyapatite in volume and adjusted to pH 5.0 using NaOH [14]. This procedure resulted in lesions approximately 35-50 µm in depth.

### Study Design

The specimens were randomly divided into four groups (10 specimens/group) according to the treatment materials. The cycling schedule was designed to approximate the pH dynamics of the oral environment and used the modified regime reported by Dunipace et al. [15]. The demineralization and remineralization cycles consisted of the episodes shown in Table 1. In order to simulate the daily acid challenges occurring in the oral cavity each cycle involved 3 h of demineralization. The specimens were kept in artificial saliva which was consisted of methyl-p-hydroxy benzoate, 2.00 g/l; sodium-carboxymethyl cellulose, 10.0 g/l; KCl, 8.38 mM; $MgCl_2.6H_2O$, 0.29 mM; $CaCl_2.2H_2O$, 1.13 mM; $K_2HPO_4$, 4.62 mM; $KH_2PO_4$, 2.40 mM; the pH was adjusted to 7.0 using KOH and no precipitation was observed in the solution during the experimental period [16]. The regimen was repeated for 21 days. The artificial saliva was changed daily and the treatment materials were freshly prepared in every application. All the time except applications, the samples were kept in artificial saliva that was mixed by a magnetic stirring machine (Multipoint HP15P, Variomag, USA).

Treatment Materials: NaF dentifrice, Ipana consisted of 1450 ppm fluoride and was used as a positive control group. CPP-ACP agent, Tooth Mousse in the paste form and hydroxyapatite agent, Remin Pro (containing 1450 ppm fluoride) in the paste form were applied on the specimens as instructed by manufacturers except application times. All

treatment materials except fluoride varnish, Pro fluorid, applied three times daily, the varnish was applied for 1 minute once a week for three weeks.

| Time | Application |
|---|---|
| 08:00 – 09:00 | Lacticacid |
| 09:00 – 09:01 | Treatmentmaterials |
| 09:01 – 13:00 | Artificialsaliva |
| 13:00 – 14:00 | Lacticacid |
| 14:00 – 14:01 | Treatmentmaterials |
| 14:01 – 19:00 | Artificialsaliva |
| 19:00 – 20:00 | Lacticacid |
| 20:00 – 20:01 | Treatmentmaterials |
| 20:01 – 08:00 | Artificialsaliva |

**Table 1:** The pH-cycling model used in this experiment (Dunipace et al. [15]).

### Assessment of mineral content-fluorecam& QLF

Assessments of the mineral content of the demineralized area of each specimen were obtained before and after each test period using both the FluoreCam and QLF systems. Using the FluoreCam System the images were acquired with and without dehydration for 5 seconds and analyzed using the specially designed software. The parameters to be assessed included ΔF: % fluorescence loss, and lesion area (mm2) and ΔQ: lesion volume (% x mm2, florescence loss times the area). Any significant change in fluorescence indicated that remineralization (or demineralization) has taken place.

### Surface micro hardness measurements

Following the lesion formation, the surface micro hardness of the lesioned portion of the specimens was determined using micro hardness tester (Leco LM247AT micro hardness tester, Cedex, France). The parameters were 200g force, for 15 seconds with a Vickers indenter. Four indentations were made on each specimen (one in each quadrant) and averaged for an average specimen value (kgf/mm$^2$). This provided a baseline surface hardness value. By performing the indentations prior to the Poly Crystalline Diamond inserts (PCDs) assessments, the indentation marks were in both the pre-test and the post-test determinations.

Following the removal of the specimens, they were individually mounted on plexiglass rods in as flat of an orientation as possible so post-test hardness and mineral content determinations can be made. After the post-test PCDs measurements, the post-test surface micro hardness indentations were made in the same manner as described above. Any significant increase in hardness over the test period was indicative of remineralization and any significant softening was indicative of demineralization.

### Statistical analysis

To compare the remineralization effects of the treatment materials used in each group, repeated measures ANOVA tests were conducted, with Tukey's multiple comparison tests for post hoc analysis. All statistical analyses were carried out using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL), with a level of 0.001 considered as significant.

## Results

Through the fluorescence assessments; both FluoreCam and QLF Systems, although all samples resulted in remineralization there was no statistical difference between all groups (p>0.001). The mean ΔF (fluorescence loss) and ΔQ (lesion volume) values (± SD) for FluoreCam System are; Ipana 3.53 ± 2.05 and 7.75 ± 5.03, Tooth Mousse 8.24 ± 1.22 and 26.10 ± 3.83, Remin Pro7.67 ± 1.88 and 22.80 ± 4.95, Pro fluorid varnish 10.03 ± 1.34 and 24.48 ± 3.76 (Table 2). Although all samples resulted in remineralization, there was significant difference between Ipana and the tests groups (Remin Pro, Pro fluorid, Tooth Mousse). However there was no statistical difference among the test groups with FluoreCam System (p>0.001).

| Group | FLUORECAM | | QLF | |
|---|---|---|---|---|
| | ΔF (%) | ΔQ (% x mm$^2$) | ΔF (%) | ΔQ (% x mm$^2$) |
| Ipana | 3.53 ± 2.05 | 7.75 ± 5.03 | 1.01 ± 1.22 | 15.25 ± 8.75 |
| Tooth Mousse | 8.24 ± 1.22 | 26.10 ± 3.83 | 2.08 ± 0.65 | 21.24 ± 3.92 |
| Profluorid | 10.03 ± 1.34 | 24.48 ± 3.76 | 1.32 ± 0.99 | 2.22 ± 5.50 |
| Remin Pro | 7.67 ± 1.88 | 22.80 ± 4.95 | 1.94 ± 0.73 | 22.22 ± 5.75 |

**Table 2:** Fluorescence assessments; ΔF and ΔQ values of obtained data in the study.

* Mean ± SEM

The mean ΔF and ΔQ values (± SD) for QLF are; Ipana 1.01 ± 1.22 and 15.25 ± 8.75 Tooth Mousse 2.08 ± 0.65 and 21.24 ± 3.92, Remin Pro 1.94 ± 0.73 and 22.22 ± 5.75, Pro fluorid varnish 1.32 ± 0.99 and 2.22 ± 5.50 (Table 2). All groups showed remineralization Tooth Mousse (Recaldent, GC Europe, Ham burg, Germany) and Remin Pro had higher value than Ipana while Pro fluorid was had lower value. However there was no significant difference between the groups.

However microhardness outcomes indicated significant differences; the mean ΔVHN values for surface microhardness are Ipana 6.76 ± 1.96, Tooth Mousse -9.45 ± 1.50, Remin Pro 16.53 ± 1.50 and Pro fluorid varnish -9.67 ± 1.79 (Table 3). NaF toothpaste and ReminPro both provided remineralization as Remin Pro showed high value (p<0.001). Tooth Mousseand Pro fluorid did not differ significantly (p>0.001) and showed decreased demineralization (Table 3).

## Discussion

One of the purposes of this study was to evaluate the effects of different remineralization treatments after the formation of artificial caries-like lesion in enamel in an attempt to mimic the oral environmental condition.

Fluoride is the proven most popular agent for the caries prophylaxis. NaF has been the most approved effective

| Group | Microhardness (kgf/mm²) | FluoreCam ΔQ (% x mm²) | QLF ΔQ (% x mm²) |
|---|---|---|---|
| Ipana | 6.76 ± 1.96 | 7.75 ± 5.03 | 15.25 ± 8.75 |
| Tooth Mousse | -9.45 ± 1.50 | 26.10 ± 3.83 | 21.24 ± 3.92 |
| Profluorid | -9.67 ± 1.79 | 24.48 ± 3.76 | 2.22 ± 5.50 |
| Remin Pro | 16.53 ± 1.50 | 22.80 ± 4.95 | 22.22 ± 5.76 |

**Table 3:** Statistically significant differences of the obtained results in the study.

* Mean ± SEM

remineralization agent used as dentifrice demonstrated a positive result at all test methods in this study (Table 3). The other source of prophylaxis methods is the varnish with high concentrations of fluoride. There are many studies reporting the success of varnishes that remineralize the incipient caries lesions. The varnish, Pro fluorid, demonstrated demineralization's lightly persistent on white spot lesion in this study. The reason could be too high viscosity of this varnish. This may result with the shallow bond between the enamel surface and the varnish; the bond could be broken during the pH cycling process. Actually the obtained result with varnishes may be inadequate time till hardening for fluoride to bond into crystallites under the conditions ph cycling regimen used in this study.

The other agent, CPP-ACP's rematerializing potential has been shown at previous studies [4,17,18]. There were unexpected results with the CPP-ACP in our study contrary to those previous reported researches. The application time of the samples was processed as one minute in the present study although the recommended period was three minutes from the manufacturer Company. The reason that one minute application period was planned to set up similar period of time for all the test materials. Although QLF and FluoreCam Systems were still positive, micro hardness showed negative results pointing the presence of untreated demineralization. The reason for this demineralization was probably due to short application time used in this study. Although there are some other studies showing the same results with the present study that application time (2-3 weeks, 2-3 application for 2-3 minutes) were not enough for the remineralization process, it may be right to apply each materials recommended time for the process [19-22]. In addition to the short application time, another reason for this phenomenon probably was the small number of indentations (4) made on the samples. It is suggested that at least 10 indentations should be made on each sample in the future studies.

Remin Pro is relatively a new agent for remineralization process. More remineralization was obtained with this agent than the control agent in this study. Probably both ingredients in this treatment agent, fluoride and hydroxyapatite enhanced the remineralization by infiltrating through the lesion body and precipitating on the surface, obtaining more remineralization on the surface (Tables 2 and 3).

The major interactions of the enamel with the oral environment occur on the surface layer of the samples [23]. Test methods used were fast and easy-to measure non-destructively reflecting the mineral changes. They also allow repeated measurements of the same specimen over a given period of time reducing the experimental variation.

Although there are some factors confining its success such as stain [24], saliva [16], dehydration [25] and angulation [26,27], QLF has shown great promise as an early caries detection method. FluoreCam (Thereametric Co., Indianapolis, USA) is a new device that has similar principles as QLF but each system's image capturing and data analysis functions operate differently. These differences define the clarity of images, accuracy of follow-up images from visits and selection of the same area for analysis. The FluoreCam System is easier to determine the suspected area by selecting de- and remineralized areas and measuring by the software automatically. In addition FluoreCam System displays the actual baseline image on the screen and enables the examiner take accurate images. In the present study both fluorescence assessments were found with no differences between test groups. All test groups showed similar remineralization process in this study. The reason for the demineralization that was found with micro hardness assessment might be; both of the florescence methods might have detected the remineralization on thesurface of thelesion but under this remineralized surface may be there was still demineralized area left. Thus we might detected demineralization with micro hardness assessment.

## Conclusions

The application of Remin Pro, Tooth Mouse, Ipana and Pro fluorid showed remineralization potential on 35-50µm enamellesion by QLF and FluoreCam systems under the conditions of this study.

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