Scientific Article

# Treatment of fluorosed and white-spot human enamel with calcium sucrose phosphate in vitro

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## Abstract

*A number of treatments have been devised to improve the appearance of fluorosed enamel. However, many of these have been empirically based, and the success of the various treatment regimens have not been quantitated. In this study, the relative whiteness of normal, mildly fluorosed, moderately fluorosed, and carious white-spot lesions on extracted teeth was quantitated by light reflectance using a Minolta Chroma Meter. The color was again determined following a number of treatment regimens to assess the potential use of various agents in treating the enamel lesions. Treatment of the enamel with a 35% hydrogen peroxide gel resulted in a significantly increased whitening, which was not reduced by subsequent treatment ($P < 0.05$). Removal of the enamel surface with a dental bur, followed by treatment with 5.25% sodium hypochlorite and placement in an artificial saliva was successful for returning white-spot lesions to a normal enamel color. Treatment of enamel with 5.25% sodium hypochlorite followed by calcium sucrose phosphate paste and placement in artificial saliva was most successful in returning both white-spot and fluorosed lesions to a normal color. SEM imaging of the calcium sucrose phosphate treated enamel suggests that this treatment filled the porous enamel, resulting in a normal light reflectance from the enamel. (Pediatr Dent 17:340–45, 1995)*

Enamel fluorosis is a porosity in the enamel resulting from exposure of the developing tooth to above-optimal levels of fluoride. The porosity is subsurface to a well-mineralized surface layer in all but severely fluorosed enamel, where the surface layer is broken down, exposing the porous enamel directly to the oral environment. The degree of porosity and the depth of the lesions varies depending on the fluoride exposure. The chalky appearance of fluorosed teeth reflects the degree of porosity, which can be as much as 25% pore volume and may extend to the dentinoenamel junction in severely fluorosed teeth.[1]

Several studies have suggested that mild fluorotic lesions repair with time. This suggests a decrease in subsurface porosity, possibly by remineralization through long-term exposure to saliva.[2] Indeed, some of the proposed empirical treatments to improve the esthetics of fluorosed enamel may act to remove the outer enamel surface and allow more rapid subsurface mineralization of the fluorotic lesion through saliva exposure.

A number of these treatments to improve the esthetics of discolored or fluorosed teeth have been described in the clinical literature. These include rubbing the teeth with 18% hydrochloric acid both with and without heat,[3,4] and treating with hydrogen peroxide with or without heat.[5–8] Techniques using hydrochloric acid combined with hydrogen peroxide or heat have been described.[9] Croll[10] suggested that all these techniques could be enhanced by microabrasion or surface layer removal. Pretreating the enamel with sodium hypochlorite followed by dental adhesive resin has been suggested for severely fluorosed teeth.[11]

Calcium sucrose phosphate paste (CaSP) as a treatment for fluorosed enamel first was reported in 1982 by Powell and Craig.[12] The treatment protocol was to first pumice the teeth, then to apply a 2% sodium fluoride solution followed by a thick layer of 40% CaSP gel. The rationale for first applying a fluoride solution and then applying CaSP was not provided. A substantial improvement in esthetics was reported, based on subjective viewing of photographs taken before and after treatment. In 1986 Myers and Lyon[13] used a different protocol. Teeth were pumiced, etched with 37% phosphoric acid for 2 min, a 2% sodium fluoride was applied for 4 min, and finally a thick layer of 40% CaSP gel was applied for at least 30 min. The patient was advised to rinse at home with sodium monofluorphosphate solution. A rationale for this protocol was not provided, but the reported results were similar to those reported by Powell and Craig.[12]

Though most of these reports are case reviews documented by photographs, some of the treatments appear promising for the esthetic improvement of enamel opacities. In particular, the use of CaSP appears to be a potentially effective approach. In this study we have quanti-

tated the change in appearance of fluorotic and white-spot lesions in extracted teeth before and after various treatments, using a colorimeter to measure the surface color reflectance of the enamel. Colorimeters have been used successfully to study the color of ceramics and the color changes of teeth before and after bleaching with hydrogen peroxide.[14,15] Therefore the use of a colorimeter in this study allowed an objective determination of the color of fluorosed and white-spot enamel lesions following treatment, relative to normal enamel.

## Materials and methods

### Sample collection

A total of 177 human teeth were collected; 62 with various degrees of fluorosis were from Colorado Springs, Colorado (water supply containing 2–3 ppm F). Sixty-nine teeth with normal macroscopic appearance were collected in Boston, Massachusetts. Forty-six teeth with various white-spot lesions, some with severe decalcification around orthodontic brackets, also were collected from the Boston area. All teeth were lightly cleaned with flour of pumice and stored in a moist atmosphere at 4°C until use. The fluorosed teeth were classified into three general categories of mildly, moderately or severely fluorosed according to Dean's index.[16] Teeth with severe fluorosis, resulting in a darkening of the enamel were excluded from further study of treatment regimens.

### Characterization by measurements of light reflectance

Initial photographs were taken of all teeth, and the whiteness of the enamel lesions was quantitated by measuring color reflectance using a Minolta Chroma Meter CR241 (Minolta, Ramsey, NJ). To standardize measurements, enamel blocks containing the lesion of interest were cut using a diamond dental bur without disturbing the outer enamel surface. A 4-mm-diameter window was made by placing a circular piece of parafilm of the same diameter over the lesion, and covering it and the surrounding enamel with Delton light cured pit and fissure sealant (Johnson and Johnson, New Brunswick, NJ). After polymerization of the sealant, the parafilm was carefully removed, revealing the enamel lesion.

Light surface reflectance measurements were expressed in L*a*b* color space measurements established by the Commission de L'Eclairage in 1978,[17] and are related to human color perception in all three color dimensions. L* values represent color gradients from white to black, a* values represent color gradients from green to red, and b* values represent color gradients from blue to yellow. Only L* value measurements of light surface reflectance were used in this study with whiter colors having a higher reading, and darker colors a lower reading.

To ensure a reproducible position of each enamel lesion with regard to the Chroma Meter, a wax mold for each block was made and stored for future use. The reproducibility of the method was tested by making 10 subsequent L* measurements on one lesion. The L*-value of the enamel was measured, the block with its wax base was removed from the stand, and then was replaced for a second measurement. This was repeated 10 times, with a standard error of measurement of 0.9%, showing high reproducibility of the measurement process. Preliminary studies showed that L* values did not change when the teeth were dry versus wet, so for convenience all measurements were done with the teeth dry.

### Treatment protocols

The teeth were divided into three groups including normal, fluorotic (both mild and moderately fluorosed teeth), and white-spot lesions secondary to orthodontic treatment. The effects of various treatments on these teeth have been grouped as: 1) use of sodium hypochlorite, 2) use of hydrogen peroxide, 3) use of calcium sucrose phosphate. Teeth were placed into treatment groups of 4–10 teeth. Due to constraints on the number of available teeth, teeth with white-spot lesions were not measured for a color change following exposure to artificial saliva alone (group 1a). Initial studies were done with 10 teeth per treatment group. However, the desirability of further treatment protocols combined with a limited number of available teeth resulted in fewer teeth per treatment group in the later studies, with a final variability in the number of teeth per treatment group.

In treatment group 1, the effect of sodium hypochlorite on fluorosed and white-spot enamel was determined. In group 1a, teeth were immersed in artificial saliva for 24 hr. In group 1b, 5.25% sodium hypochlorite was placed on the enamel window for 20 min, and the enamel was then rinsed with deionized water. Teeth in group 1c were treated the same as in group 1b with additional immersion of the tooth in artificial saliva for 24 hr. In group 1d, the surface of the enamel was removed by mechanical abrasion using a dental green stone, followed by a 20-min exposure to 5.25% sodium hypochlorite, a rinse with deionized water, and placement in artificial saliva for 24 hr. The ion concentration in the artificial saliva was similar to that found in whole saliva[18,19] and was composed of 0.8 mmol/L calcium, 3 mmol/L $PO_4$, 150 mmol/L KCl, and 20 mmol/L cacodylate buffer at pH 7.

In treatment group 2, the effect of hydrogen peroxide on fluorosed and white-spot enamel was determined. The exposed enamel lesions were: in group 2a, covered with a 35% hydrogen peroxide gel (Starbrite bleaching system, Stardent Laboratories, Midvale, UT) for 20 min and rinsed with deionized water; in 2b, treated as above followed by immersion in artificial saliva for 24 hr.

The effect of calcium sucrose phosphate paste (CaSP) on enamel lesions was determined in treatment group 3. The CaSP paste (marketed as a toothpaste for

dentin sensitivity, under the trade name *Fluoran*, (Creighton Pharmaceuticals, Sidney, Australia) was used as a source of CaSP. This paste contained 10% CaSP/calcium orthophosphate complex. In group 3a, CaSP was applied to the teeth and rubbed into the enamel with a cotton pellet for 1 min, followed by a 24-hr exposure to artificial saliva. The enamel from group 3b was exposed to 35% hydrogen peroxide for 20 min, rinsed with deionized water, and treated with CaSP as described above, followed by a 24-hr exposure to artificial saliva. In group 3c, the enamel was exposed to 5.25% sodium hypochlorite for 20 min, rinsed with deionized water, and treated with CaSP as described above, followed by a 24-hr exposure to artificial saliva.

During these procedures, only the circular experimental windows were exposed, with the remaining surfaces of the blocks protected by dental sealant and dental wax. Blocks were suspended from a wax-covered glass rod in a capped test tube, so that the window on the enamel block was totally exposed to the artificial saliva. The significance of the differences between the means of the control (normal) and experimental groups was evaluated using a two-tailed Student's *t*-test for unpaired samples. The paired test was used to compare pre- and post-treatment L*-values. Finally, post-treatment L* values were compared with the control L*-value (normal group) with an unpaired two-tailed Student's *t*-test to determine the effectiveness of the various treatments. Values of $t$ indicating $P < 0.05$ were regarded as statistically significant differences.

### Scanning electron microscopy

Three fluorosed and two normal teeth treated with CaSP paste were prepared for SEM. Grooves were scored with a diamond disc on either side of the window, then fractured either at room temperature or under liquid nitrogen with a chisel. This procedure resulted in relatively flat fractured surfaces, which are necessary for comparative elemental analysis. The fractured surfaces were mounted parallel to the surface of 1-cm-diameter carbon stub with carbon paint, and were then coated with a thin layer of carbon in a JEOL 4 vacuum evaporator. Specimens were viewed in a JEOL 6400 SEM (JEOL, Peabody, MA). Samples were imaged in both secondary (SEI) and back-scattered (BEI) electron modes.

## Results

### Color measurement

The initial L*-values for the different groups were all significantly different from that of normal enamel. The L*-value for mildly fluorosed teeth (N = 28) showed a whiter color, as indicated by a significantly higher L* value of 78 ± 3.9, compared with normal teeth (N = 56), which had an L*-value of 69 ± 2.9. Likewise, moderately fluorosed teeth (N = 28) were whiter with an L*-value of 83 ± 3.5, compared with normal and mildly fluorosed teeth. The severely fluorosed teeth (N = 6) were darker with a lower L*-value of 58 ± 6.4 compared with normal, mildly, and moderately fluorosed teeth. The whiteness of the enamel for the white-spot lesions (N = 36) was L* = 80 ± 3.5, which was similar to the color of moderately fluorosed teeth.

The effects of the various treatment protocols on the L*-values are shown in Tables 1, 2, and 3. Use of artificial saliva alone had no effect on the enamel color of fluorosed teeth. When the enamel was treated with sodium hypochlorite in combination with immersion in artificial saliva or with mechanical removal of the enamel surface (Table 1), there was no effect on the color of the fluorosed enamel. Treatment with hydrogen peroxide (Table 2) caused a significantly increased whitening (higher L*-values) of the enamel in all groups. The whitening effect of peroxide remained in teeth that were subsequently placed in artificial saliva and in teeth that were subsequently treated with CaSP (Table 3). Use

**Table 1. Treatment group 1: use of sodium hypochlorite**

*Mean L*-values (SD)*

*a) Artificial saliva (no hypochlorite)*

| | *Normal* | *Fluorotic* | *White Spot* |
|---|---|---|---|
| | N = 5 | N = 6 | |
| pre- | 68.1 (2.8) | 75.9 (1.8)• | |
| post- | 69.4 (2.2) | 74.8 (1.7)• | |

*b) Sodium hypochlorite (alone)*

| | *Normal* | *Fluorotic* | *White Spot* |
|---|---|---|---|
| | N = 10 | N = 10 | N = 10 |
| pre- | 70.0 (3.3) | 81.4 (3.9)• | 82.7 (3.0)• |
| post- | 69.5 (3.1) | 79.9 (3.7)• | 80.9 (2.8)• |

*c) Sodium hypochlorite followed by artificial saliva*

| | *Normal* | *Fluorotic* | *White Spot* |
|---|---|---|---|
| | N = 7 | | N = 4 |
| pre- | 70.5 (2.5) | | 82.5 (0.9)• |
| post- | 69.0 (1.4) | | 81.5 (1.5)• |

*d) Surface abrasion, sodium hypochlorite followed by artificial saliva*

| | *Normal* | *Fluorotic* | *White Spot* |
|---|---|---|---|
| | N = 7 | N = 6 | N = 4 |
| pre- | 68.9 (2.6) | 84.3 (5.7)• | 83.9 (2.1)• |
| post- | 70.2 (2.1) | 82.2 (4.2)• | 68.7 (0.8)† |

• L-value is significantly different from the L-value for normal enamel ($P < 0.05$).

† Post-treatment value is significantly different from pre-treatment value ($P < 0.05$).

**TABLE 2. TREATMENT GROUP 2: USE OF HYDROGEN PEROXIDE**

*Mean L*-values (SD)*

| | *Normal* | *Fluorotic* | *White Spot* |
|---|---|---|---|
| *a) Hydrogen peroxide (alone)* | | | |
| | N = 10 | N = 10 | N = 4 |
| pre- | 68.6 (2.3) | 81.12 (3.8)• | 81.3 (0.6)• |
| post- | 74.9 (2.6)•† | 88.2 (3.1)•† | 86.9 (1.0)•† |
| *b) Hydrogen peroxide followed by artificial saliva* | | | |
| | N = 4 | N = 6 | N = 6 |
| pre- | 69.1 (2.6) | 78.8 (2.5)• | 77.1 (2.7)• |
| post- | 76.8 (2.5)•† | 83.3 (1.7)•† | 80.9 (2.2)• |

• L*-value is significantly different from the L*-value for normal enamel ($P < 0.05$).

† Post-treatment value is significantly different from pre-treatment value ($P < 0.05$).

**TABLE 3. TREATMENT GROUP 3: USE OF CALCIUM SUCROSE PHOSPHATE**

*Mean L*-values (SD)*

| | *Normal* | *Fluorotic* | *White Spot* |
|---|---|---|---|
| *a) Calcium sucrose phosphate followed by artificial saliva* | | | |
| | N = 6 | N = 6 | N = 4 |
| pre- | 70.1 (3.0) | 79.1 (6.6)• | 83.0 (2.4)• |
| post- | 69.5 (1.6) | 72.8 (2.7)† | 75.6 (1.8)† |
| *b) Hydrogen peroxide, calcium sucrose phosphate followed by artificial saliva* | | | |
| | N = 10 | N = 10 | N = 4 |
| pre- | 68.6 (2.3) | 81.16 (3.82)• | 81.26 (0.65)• |
| post- | 72.4 (1.3)•† | 84.27 (3.23)• | 86.16 (2.65)•† |
| *c) Sodium hypochlorite, calcium sucrose phosphate followed by artificial saliva* | | | |
| | N = 10 | N = 10 | N = 10 |
| pre- | 70.1 (3.3) | 81.4 (4.0)• | 82.7 (3.1)• |
| post- | 69.7 (2.9) | 70.6 (3.40)† | 70.7 (4.9)† |

• Value is significantly different from the L*-value for normal enamel ($P < 0.05$).

† Post-treatment value is significantly different from pre-treatment value ($P < 0.05$).

of CaSP paste either alone or with sodium hypochlorite on the enamel surface produced a significant decrease in whiteness (lower L*-value) of fluorotic enamel to result in a color similar to normal enamel (Table 3).

Unlike fluorosed enamel, the color of white-spot lesions returned to that of normal enamel following mechanical surface removal, sodium hypochlorite, and artificial saliva. A treatment group using mechanical surface removal alone was not included, so the relative importance of the sodium hypochlorite in this treatment regimen can not be determined. The use of CaSP on white-spot lesions prior to placement in the artificial saliva resulted in some color change with a significantly reduced L*-value. However, pretreatment with sodium hypochlorite prior to use of CaSP and immersion in artificial saliva was more effective in returning the color of both fluorosed and white-spot lesions to that of normal enamel.

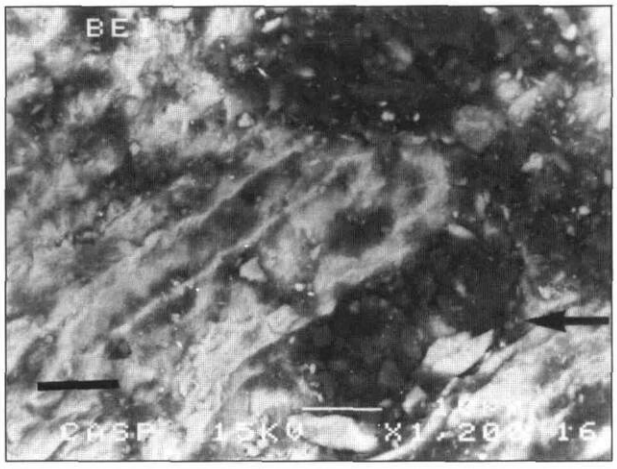

**Fig 1. Back-scattered electron image of moderately fluorosed human enamel after treatment with sodium hypochlorite, calcium sucrose phosphate paste and artificial saliva. A globular material consisting of large granules (arrow) is compacted into the enamel rods. Bar = 10 μm.**

### SEM observations

Both scanning and back-scatter electron imaging of normal enamel treated with CaSP paste, showed a regular appearance of enamel rods. However, treatment of moderately fluorosed teeth with CaSP resulted in the appearance of an irregular-shaped globular material compacted between regular enamel rods (Fig 1). In scanning and in back-scattered mode it was evident that this material was located between the enamel rods below the enamel surface, and was not merely an artifact from surface contamination.

## Discussion

The appearance of white-spot lesions and fluorotic enamel results from a subsurface porosity in the enamel below a well mineralized surface layer.[20,21] This subsurface porosity, which increases with increasing severity of fluorosis,[22] results in a whiter appearance to the enamel lesion. In this study, fluorosed and normal enamel were characterized in vitro by measuring the surface color reflectance of the enamel. The measurement of surface color reflectance showed that the enamel whiteness, as measured by the L*-value, could be directly correlated with the visual assessment of teeth as being fluorosed or having white spots. The

sensitivity of the color measurement technique is shown by the significant increase in L*-values of moderately fluorosed enamel compared with mildly fluorosed enamel. With increasing fluorosis enamel becomes more porous. Therefore, the increased whiteness (higher L*-values) of the moderately fluorosed teeth is likely related to the increased enamel porosity in these teeth. Quantitative evaluation of the color of both fluorosed and white-spot enamel using the Minolta Chroma Meter proved to be a sensitive method showing significant differences in the color of fluorosed and white-spot lesions, compared with unaffected enamel. This quantitative measurement of the whiteness of a lesion allowed an objective evaluation of the effect of various treatment regimens in altering the color of fluorosed and white-spot enamel.

The rationale for treating fluorosed enamel with oxidizing agents such as hydrogen peroxide and sodium hypochlorite was that removing surface organic material could result in a more ready diffusion of calcium and phosphate into the enamel. This increased diffusion of mineral into the lesion may increase remineralization within the subsurface lesion. Enamel was treated with both artificial saliva and deproteinizing agents (hypochlorite and peroxide) both together and separately to determine the unique contribution of each to changes in the enamel.

Treating the enamel with hydrogen peroxide was found to cause significant whitening of all teeth. This whitening effect was unchanged by any subsequent treatment regimens. Although a whitening effect may mask the fluorotic lesion by causing the less fluorotic enamel to become whiter, it creates a color that is lighter than the normal range of human tooth enamel. It therefore does not appear to be indicated for treatment of fluorotic or white-spot lesions.

Unlike hydrogen peroxide treatment, treating the enamel surface with a 5.25% sodium hypochlorite resulted in no changes in the color of either fluorosed or white-spot lesions. Previous studies have shown hypochlorite to be an effective deproteinizing agent and to increase penetration of mineral into carious enamel lesions.[23, 24] In these studies, use of sodium hypochlorite to remove the organic surface layer was effective in treating enamel lesions in combination with CaSP.

Mechanical removal of the tooth surface appeared to be effective for changing the color of white-spot lesions into the range of normal enamel. This may be due to the shallow subsurface nature of this lesion. When the highly mineralized surface area of these lesions is mechanically removed, the underlying shallow lesion may be remineralized in the artificial saliva. It is likely that removing the surface enamel through use of a dental bur or by "microabrasion" also would be effective in very mildly fluorosed teeth, where the subsurface porosity is minimal and is limited to the outer 50–100 μm of enamel surface. However, in moderately fluorosed teeth, where the porosity is greater, simply removing the surface without further "filling in" of the pores is not likely to be as effective. This suggests that carefully assessing the severity of the lesion is important prior to treatment.

Using CaSP alone decreased L*-values toward values comparable to normal enamel for both fluorosed and white-spot enamel without affecting normal tooth color. However, pretreatment with sodium hypochlorite, followed by CaSP and exposure to an artificial saliva was the most successful treatment protocol, resulting in L*-values similar to normal enamel for both fluorosed and white-spot lesions.

CaSP is a fine white powder with a bland, neutral taste. It is a mixture of calcium sucrose mono- and diphosphates, disucrose monophosphate, and inorganic calcium orthophosphate, containing approximately 11% calcium, 9.5% organic phosphorous, and 2.5% inorganic phosphorous. Prior to the reported use of CaSP in treatment of fluorosed enamel, it had been used as a food additive to reduce the incidence of dental caries in children,[25-27] and for desensitizing dentin.[28] High concentrations of calcium and orthophosphate ions in CaSP were cited as being responsible for the rapid remineralization of softened enamel and for desensitizing dentin. However, the mechanisms of action of CaSP appear to be poorly defined.

When moderately fluorosed teeth that had been treated with 10% CaSP paste were examined by scanning electron microscopy, a globular material with a granular appearance was found. Back-scattered scanning electron microscopy also showed electron absorbent areas of material that had been deposited within the rods. These results suggest that treatment with the CaSP paste resulted in filling the subsurface porous spaces rather than simply a remineralization effect as previously suggested. Filling the porous enamel would alter the light reflectance and hence the color of the porous fluorosed or white-spot enamel, returning the color to normal. This suggests that with more severe white opaque lesions, such as found in mildly and moderately fluorosed teeth as classified by Dean, an actual filling of the pores may necessary for an acceptable clinical result.

The quantitative results obtained in this in vitro study, along with previous reports, suggest that CaSP can be an effective treatment for enamel opacities. Further studies to compare different concentrations as well as the long-term effect of this material on tooth enamel are warranted. Although CaSP paste is not currently commercially available, it is potentially useful in further developing treatments to be used for this important aspect of esthetic dentistry.

## Conclusions

1. Changes in the appearance of enamel can be quantitated by measuring light reflectance.
2. Effective treatment of enamel lesions such as moderately fluorosed enamel differ from treatment of less porous lesions such as in very mildly fluorosed enamel or white-spot lesions.

3. Treatment of enamel with 5.25% sodium hypochlorite followed by a calcium sucrose phosphate paste was most effective in changing the appearance of fluorosed and white-spot lesions into a color range for normal enamel.

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