

Evaluation of NovaMin® as an adjunct to fluoride for caries lesion remineralization.

NovaMin Research Report

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Abstract: This study was conducted to evaluate a test dentifrice containing 5% NovaMin and Fluoride (MFP) to a commercially available dentifrice in remineralization of subsurface carious lesions in human tooth enamel.

Enamel sections from 18 extracted human teeth were prepared and sectioned into quadrants. In three sections simulated pre-caries surface lesions a depth of 100-150 µm were created using a demineralizing solution. One quadrant from each tooth was treated with an experimental NovaMin (5%) plus fluoride dentifrice, while another was treated with a commercially available dentifrice with Fluoride (MFP). Each sample (plus an untreated control quadrant) was exposed to 20 days of cycling through demineralizing solution, 3 minute dentifrice treatment, additional demineralizing and then saliva soaking. After treatments the treated quadrants were additionally sectioned to reveal a cross section of the lesion to allow for cross sectional analysis.

Evaluation and measurement was done using confocal laser scanning microscopy ("CLSM"), which is able to distinguish between sound enamel and demineralized enamel using fluorescing dye. The NovaMin dentifrice produced significantly more remineralization than did the Fluoride dentifrice. Fluoride dentifrice® reduced the lesion area by an average of 24.9% from the original lesion depth. NovaMin® decreased the lesion area by 41.9%. Results were statistically significant ($p < 0.001$).

Keywords: Remineralization,
Confocal microscopy, NovaMin

Pre-caries surface lesions on tooth enamel are considered precursors to subsurface lesions and cavities. Fluoride dentifrices have been shown in many studies to be effective at improving remineralization of subsurface enamel lesions. It is less clear, however, if Fluoride alone is effective at remineralizing surface lesions. A dentifrice which was more successful at remineralizing these surface lesions would intervene earlier in the tooth decay disease process and would, therefore, be of potentially great benefit clinically.

This study was conducted to evaluate a test dentifrice containing 5% NovaMin and Fluoride (MFP) to a commercially available Fluoride dentifrice (MFP alone) in remineralization of surface carious lesions in human tooth enamel.

METHODS AND MATERIALS

Collection and Storage of Teeth

A total of 18 teeth with intact surfaces, no carious lesions and no restorations were collected from a pool of teeth extracted in unrelated procedures at University of Florida Dental Clinics. All teeth were stored in 0.1% Thymol until processing. Teeth were cleaned of soft tissue debris and later sterilized using gamma irradiation.

Sectioning and Mounting

Each tooth was sectioned into quadrants along both mesiodistal and buccolingual planes using a Buehler Isomet® 300 Low Speed diamond-tipped saw. Sections were mounted individually in epoxy mounting resin and labeled. A 3-mm diameter standardized treatment "window" was created on each tooth section by grinding off 200 µm of surface enamel. This process removes non-homogenous outer surface layers formed through years of fluoridation and natural demin/remin cycling in the mouth. Mechanical polishing was

performed with successive 120, 400, 600 and 1200 grit paper.

Lesion Formation

Three of the four quadrant sections of each tooth sample were demineralized in a solution containing 2.2 mM CaCl₂, 2.2 mM NaH₂PO₄, 0.05 M Lactic Acid, and 0.5 ppm F⁻, adjusted to pH 4.5 with 50% NaOH. Conditions were maintained to produce uniform surface lesions with a depth of 100-150 µm.

Treatment Regimen

Two quadrant sections were treated in a pH cycling regimen including 3:1 toothpaste solutions of Fluoride dentifrice Regular and NovaMin® dentifrice respectively. Both dentifrices, nearly identical in composition, contain 1100 ppm fluoride although the NovaMin® paste contains 5 wt% NovaMin particles in place of an equivalent amount of silica abrasive. Tooth sections were immersed

cyclically in stirred treatment solutions, demineralization solution (detailed above) and Fusayama's synthetic saliva for 20 days at a temperature of 37°C (with the exception of the dentifrice solution treatment, which was done at 25°C).

The daily treatment regimen (pH cycling) included the following sequence:

Time Zero ("T0"): Demineralization solution 30 minutes

T0+0.5 hrs: Dentifrice treatment 3 minutes / distilled water wash / synthetic saliva

T0+7.5 hrs: Demineralization solution 30 minutes

T0+8.0 hrs: Dentifrice treatment 3 minutes / distilled water wash / synthetic saliva for 16 h

Cross-sectioning

After treatment, all four quadrant sections of each tooth were cross-sectioned through the treatment "window" to expose the lesion depth along the cross-sectional surface. During storage all sections were kept moist and refrigerated.

CLSM Analysis

CLSM (confocal laser scanning microscopy) was performed on 72 cross-sections (4 sections x 18 teeth) at the Oral Health Research Institute at Indiana University-Purdue University at Indianapolis (IUPUI) under the supervision of Dr. Marguerita Fontana.

Cross sectional analysis of enamel lesions with CLSM were based upon digital images taken at specified controlled conditions. Sound enamel registers near zero fluorescence (grayscale value ~ 0) and appears pitch black. Lesions slightly autofluoresce but uptake of the Rhodamine B dye (0.1 mM) allows the porous demineralized layer to fill and appear with considerable contrast. Analysis of all samples was conducted with a specially modified

Nikon microscope fitted with Odyssey confocal capability (Odyssey, Noran Instruments, Inc., Middleton, WI). The accompanied software (Metamorph® version 4.1.6, Universal Images Corp., West Chester, PA) calculates image-based parameters of selected lesion zones.

RESULTS AND DISCUSSION

The NovaMin® dentifrice was more successful at reducing lesion area than was Fluoride dentifrice for 16 out of 18 teeth (fig 3a, following page). Single tailed T-testing (assuming unequal variances) found these two groups to be significantly different ($p < 0.001$). For nearly all samples, the 20-day dentifrice treatment under pH cycling halted and reversed the caries process as evident in the reduction of the lesion area, as would be expected for fluoride-containing dentifrices. Fluoride dentifrice reduced the lesion area by an average of 24.9% from the original lesion depth. NovaMin decreased lesion area by 41.9%.

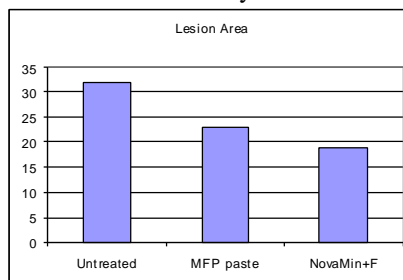


Fig 1. Cross section lesion area

Total gray value was also plotted to determine the fluorescence levels within the lesions. This parameter is commonly reported as total fluorescence because greater fluorescence corresponds to higher gray values (thus, smaller values are indicative of less porosity and dye penetration, or more mineral). The NovaMin® dentifrice reduced the total gray value more than Fluoride dentifrice for the same 16 out of 18 teeth (fig 3b, following page). Treatment groups were again found to be significantly different ($p < 0.001$). Fluoride dentifrice reduced the fluorescence (total gray value) by an average of 48.1%, NovaMin® 70.5% -- a similar ratio to the lesion area measurements.

Statistical review (SAS System software) using two-way analysis of

variance (ANOVA) of all three treatment groups yielded statistical significance ($p < 0.0001$) for both confocal parameters, lesion area and total fluorescence.

Remineralization "bands" revealing the depth at which most remineralization occurred could be easily seen in these cross sectional images (figures 2a-c). The remineralization "bands" signify mineral deposition, similar to those reported in subsurface lesion models for fluoride dentifrices. The depth of these bands could be due to several variables. A combination of diffusion rates for fluoride, calcium, and phosphates into the enamel surface in addition to twice daily acid challenge may influence band depth.

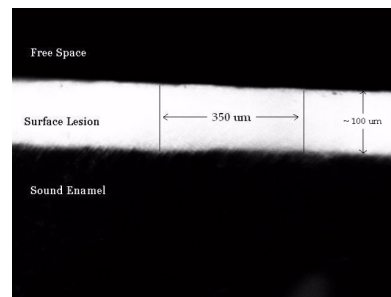


Fig 2a. Cross section of control sample

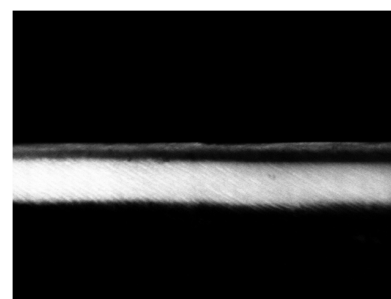


Fig 2b. Cross section of Fluoride dentifrice treated sample

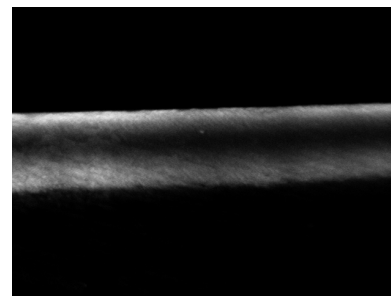


Fig 2c. Cross section of NovaMin treated sample

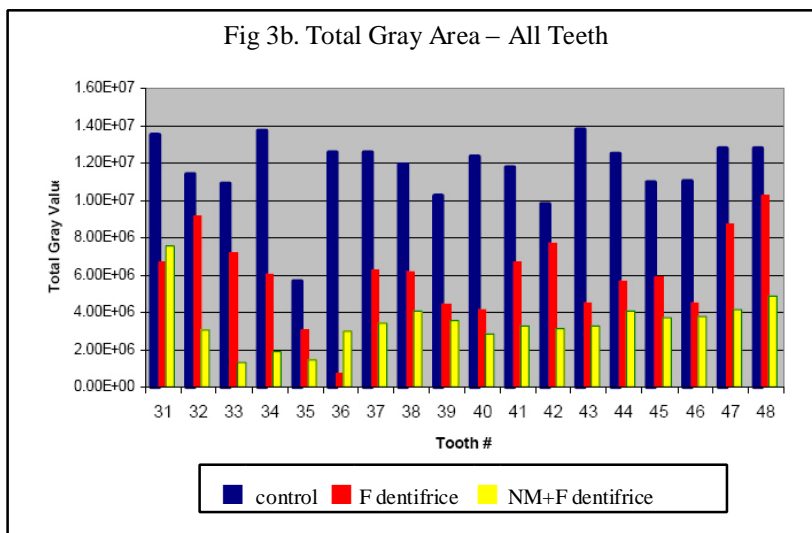
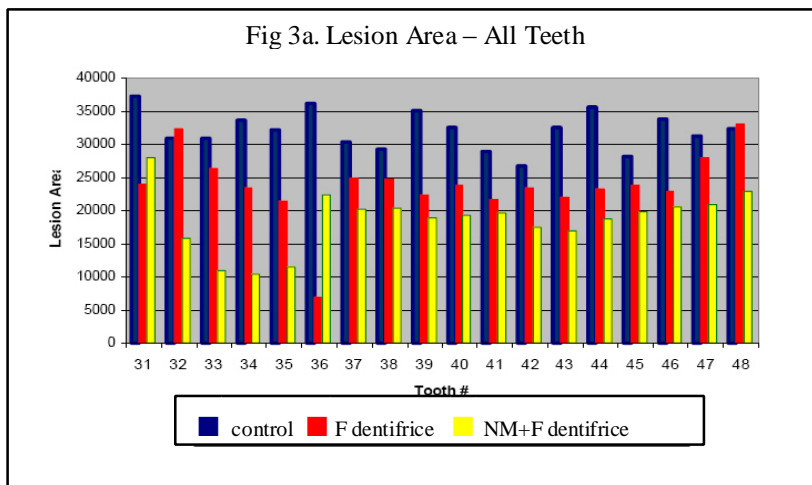
The NovaMin +MFP dentifrice resulted in a remineralization band throughout the lesion depth relative to the thinner remineralization band at the surface of the lesion for the fluoride-alone dentifrice.

The nature and degree of interaction between fluoride and NovaMin® is clearer. It appears that *in vitro*, these two agents positively interact and do not inhibit the remineralization function of the other.

Cross-sectional CLSM analysis proved to be a useful approach for surface remineralization study *in vitro*. From cross-sectional imaging it was clear that most remineralization took place at the surface during exposure to fluoride containing toothpaste. This is consistent with other published studies. Therefore data taken from the treatment surface alone would create an incomplete picture of remineralization throughout the lesion.

This study did not include organic components such as oral bacteria or plaque. Therefore anti-microbial effects were not active, and the known anti-microbial effects of NovaMin-containing dentifrices might be expected to create even larger advantage for the NovaMin dentifrice in clinical conditions. Furthermore, the key factors for remineralization in this model were ions (Ca²⁺ and PO₄³⁻) and the pH at the tooth surface. The release of these ions from NovaMin® provides a solubility gradient in favor of mineral deposition. This explains why the samples treated with NovaMin® dentifrice experienced greater remineralization, the environment was more conducive to this process. This may present a stronger case for NovaMin® dentifrice in xerostomic conditions.

carries lesions in human enamel in this *in vitro* model. This indicates that incorporation of NovaMin into fluoride dentifrices could arrest the tooth decay process earlier than currently available fluoride dentifrices.



CONCLUSION

This study has shown that NovaMin® dentifrice exhibits a greater degree of remineralization than regular fluoride dentifrice on simulated early surface