

Tubule occlusion of a NovaMin-containing dentifrice compared to Recaldent-containing dentifrice – a Remin/Demin study *in vitro*.

NovaMin Research Report

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Abstract: An *in vitro* study was conducted to determine the ability of two different materials to physically occlude dentinal tubules (SootheRx™ and MI Paste dentifrices) when repeatedly challenged with an acidic (demineralizing) environment. The active ingredients in SootheRx™ and MI Paste (NovaMin® and RECALDENT™, respectively) are both presented to consumers as sources of minerals that are naturally found in teeth. Brushing with both products should, in theory, aid in remineralizing tooth surfaces and occluding exposed tubules during the daily remineralization/demineralization (remin/demin) cycle that takes place in the mouth.

Root portions of bovine incisors were progressively ground, polished and acid-etched to expose tubules in the dentin layer. The root dentin samples were randomly assigned to one of three treatment groups: Control (brushed with DI water), MI Paste, or SootheRx™ and were subjected to the following protocol every day for a total of 10 days: 1) 30-minute soak in demineralizing solution, 2) 2-minute brush with the appropriate dentifrice, 3) 6-hour soak in artificial saliva, 4) 30-minute soak in demineralizing solution, 5) 2-minute brush with the appropriate dentifrice, 6) 15-hour (overnight) soak in artificial saliva. At the end of the 10-day remin/demin cycle the root dentin samples were prepared for analysis with scanning electron microscopy (SEM). Quantitative tubule occlusion data were independently obtained by three people who examined each SEM image and recorded the number of open, partially occluded, and total visible (open + partially occluded) tubules.

Treatment with both dentifrices significantly reduced the number of open tubules compared to the control group. The average number of open tubules visible in an SEM image (60 x 44µm) dropped from 48 in the control group to 28 in MI Paste-treated samples and less than 1 in samples treated with SootheRx™. Samples treated with MI Paste had a large proportion of partially occluded tubules and very few fully occluded tubules present on the dentin surface. The number of open, partially occluded, and total visible tubules seen in treated with SootheRx™ were significantly less than in the MI Paste-treated samples.

The current study has demonstrated that the NovaMin® particles in the SootheRx™ dentifrice are superior to the RECALDENT™ in MI Paste in depositing a mineral layer on dentin that will occlude dentinal tubules and resist demineralization by repeated acid challenges.

Introduction

Recently, a 10-day remineralization/demineralization ('remin/demin') study was conducted with the goal of comparing the longer-term remineralization benefits of SootheRx™ toothpaste with those of MI Paste. The active ingredients in SootheRx™ and MI Paste (NovaMin® and RECALDENT™, respectively) are both presented to consumers as sources of minerals that are naturally found in teeth. Brushing with both products should, in theory, aid in remineralizing tooth surfaces during the daily remineralization/demineralization cycle that takes place in the mouth. This study was

designed to test this theory using an *in vitro* model of this daily remineralization/demineralization cycle.

Materials and Methods

Preparation of Bovine Root Dentin Samples

The root portion of 15 bovine incisors were separated from the crown portion at the cementum-enamel junction (CEJ) using a diamond-embedded blade and a low-speed saw. The root portions of the teeth were progressively ground at low speeds using 320, 600, and 1200 grit silicon carbide abrasive papers to expose and polish the dentin layer. Fresh deionized (DI) water was kept

flowing on the grinding wheel during all grinding and polishing steps. The samples were ultrasonicated three times in fresh DI water for 15 minutes each. The smear layer was removed by soaking the samples in a 40% o-phosphoric acid solution for 15 minutes. The cleaning procedure was then repeated by ultrasonating the samples an additional three times in DI water for 15 minutes each. Prepared samples were refrigerated overnight in TRIS buffer before testing was performed.

Preparation of Remineralizing Solution

Artificial saliva (remineralizing solution) was prepared to have the following composition:

2.200g/L Gastric Mucin
0.381g/L NaCl
0.213g/L CaCl₂•2H₂O
0.738g/L K₂HPO₄•3H₂O
1.114g/L KCl

Each ingredient was added separately to stirring DI water and was allowed to dissolve completely before the next ingredient was added. The solution was heated to 37°C and the pH was adjusted to 7.00 using 85% lactic acid.

Preparation of Demineralizing Solution

Demineralizing solution was prepared to have the following composition:

2.2mM CaCl₂•2H₂O
2.2mM NaH₂PO₄•7H₂O
0.05M Lactic Acid
0.5ppm Fluoride

Each ingredient was added separately to stirring DI water and was allowed to dissolve completely before the next ingredient was added. The solution was heated to 37°C and the pH was adjusted to 4.52 using 50% NaOH solution.

Remineralization/Demineralization Study Protocol

The 15 root dentin samples were randomly assigned to one of three treatment groups: 1) Control (brushed with DI water), 2) MI Paste, or 3) SootheRx™ and were subjected to the following protocol every day for a total of 10 days:

08:00-08:30: All teeth soaked in demineralizing solution at 37°C

08:30-09:30: All teeth removed from demineralizing solution, brushed using a soft-bristled toothbrush with the appropriate dentifrice, and gently rinsed with deionized (DI) water

09:30-15:30: All teeth soaked in artificial saliva at 37°C

15:30-16:00: All teeth soaked in demineralizing solution at 37°C

16:00-17:00: All teeth removed from demineralizing solution, brushed using a soft-bristled toothbrush with the appropriate dentifrice, and gently rinsed with deionized (DI) water.

17:00-08:00: All teeth soaked in artificial saliva at 37°C

SEM Analysis

At the end of the 10-day study, the teeth were dried at 37°C and mounted for analysis using scanning electron microscopy (SEM). Three teeth from each treatment group were mounted on carbon mounts and coated with carbon coating and the remaining two teeth from each treatment group were mounted on aluminum mounts and coated with a gold/palladium coating. SEM images were obtained at 500X and 2000X from three randomly chosen spots on each tooth surface.

Quantitative Evaluation of Tubule Occlusion

Tubule counting was performed on all SEM images that were obtained at 2000X (15 images per treatment group). Three people separately examined each image and counted the number of open tubules and partially occluded tubules using previously agreed-upon criteria. Tubules that could not be fully visualized on the SEM images were not counted.

For each SEM image the tubule counts from the three people were averaged together to obtain the number of open, partially occluded, and total visible (open + partially occluded) tubules (n=3). For each treatment group, the data from the 15 SEM images were averaged together to obtain the average number of open, partially occluded, and total visible tubules (n=15).

Data were statistically analyzed using SigmaPlot software (v9.01, Systat Software, Inc.). Data did not have a normal distribution so the non-

parametric ANOVA on Ranks test was used to detect significance. When significance was found, pairwise comparisons were made using the nonparametric Student-Newman-Keuls test (significant if p<0.05). Data are reported as mean ± standard error of the mean.

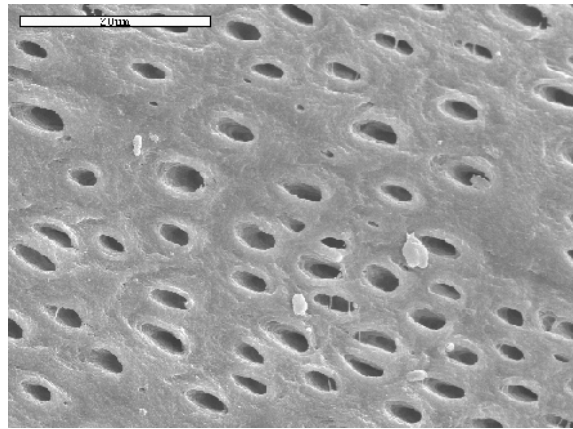
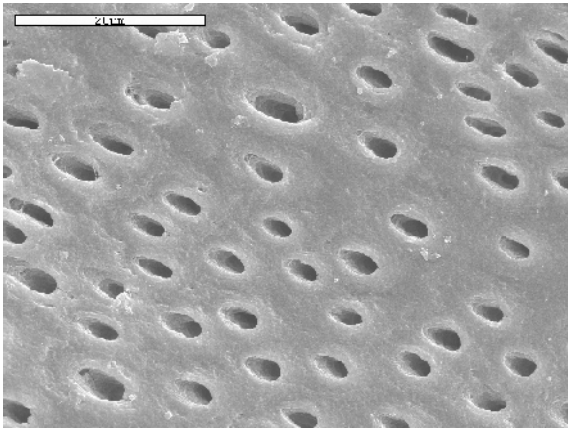
Results

Figures 1 – 3 (following page) contain representative SEM images of teeth from each of the three treatment groups. It is clear that the polished, acid-etched bovine dentin samples contain open tubules (Figs. 1a and 1b). All five control samples were very uniform in appearance and in the number of tubules present.

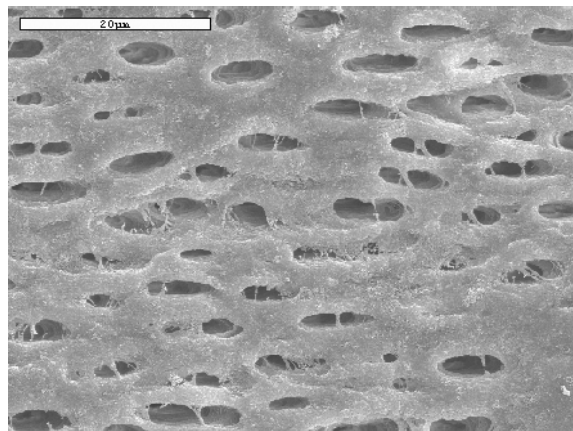
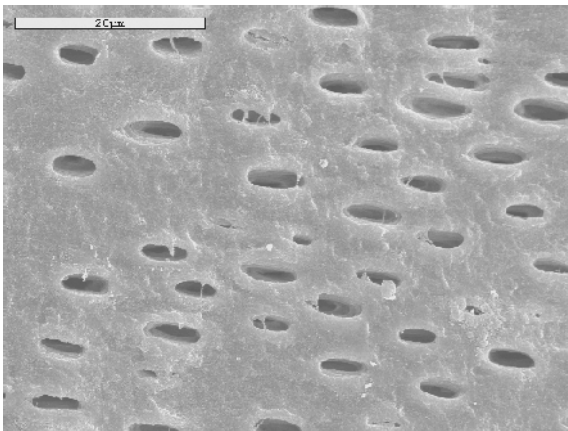
Figures 2a and 2b show the samples from the MI Paste treatment group after 10 days of remin/demin cycling. A film can be seen on the dentin and there is significantly more fibrous-appearing material on the surface compared to the control samples. There is also evidence of some tubule occlusion (top and lower right in Fig. 2a and lower portion of Fig. 2b).

Figures 3a and 3b show the SootheRx™-treated dentin samples after 10 days of remin/demin cycling. Most of the exposed dentin tubules have been occluded and there is significant build-up of a reaction layer on the surface of the dentin.

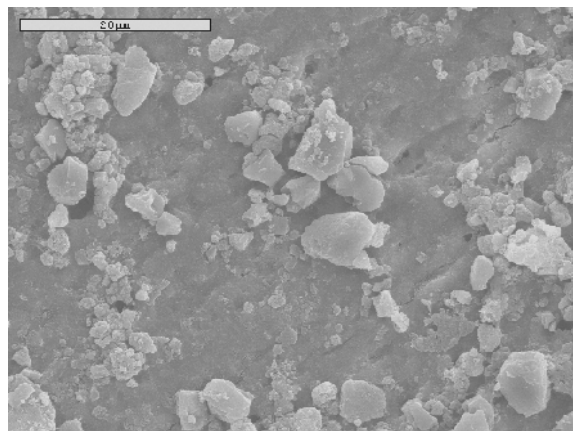
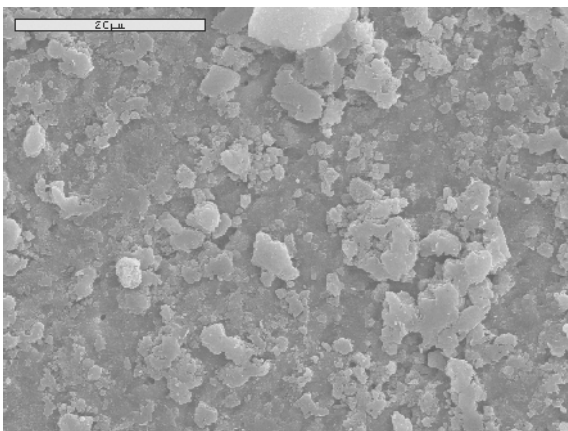
Results



Figures 1a and 1b: SEM Images of Control Teeth Brushed with DI Water (2000X)



Figures 2a and 2b: SEM Images of Teeth Brushed with MI Paste (2000X)



Figures 3a and 3b: SEM Images of Teeth Brushed with SootheRx™ (2000X)

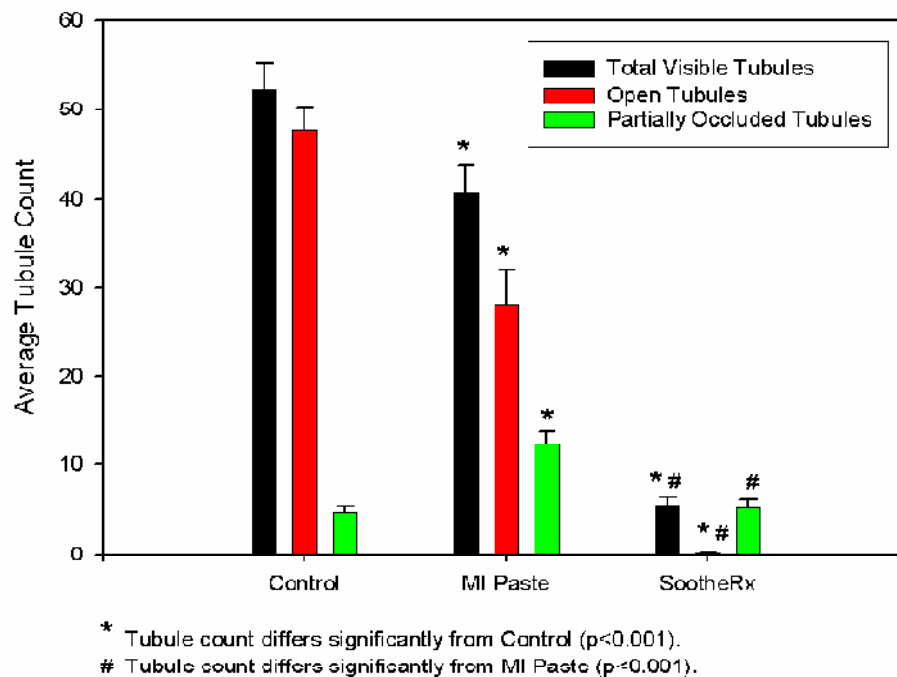


Figure 4: Quantitative Tubule Occlusion Data

Figure 4 contains the results of the quantitative evaluation of tubule occlusion after 10 days of remin/demin cycling. The samples from the MI paste and SootheRx™ treatment groups had significantly less total visible and open tubules than the control samples. There were more partially occluded tubules visible on the MI Paste-treated samples than the control samples while the SootheRx™-treated samples contained the same number of partially occluded tubules as the control group. The number of open, partially occluded, and total visible tubules seen in samples from the SootheRx™ treatment group were significantly less than seen in the MI Paste-treated samples.

Discussion

The use of *in vitro* remineralization/demineralization methods has been well accepted by the dental community in assessing the efficacy of materials to physically occlude tubules and to remineralize the surface of both dentin and enamel. In this study, two products that claim to treat dentine hypersensitivity were compared side-by-side in a 10 day remineralization/demineralization protocol. The qualitative and quantitative results presented here clearly demonstrate that the reactivity of the NovaMin® particles in the

SootheRx™ dentifrice occluded the vast majority of dentin tubules and was much more effective than the competitive MI Paste.

While a definitive explanation for the observed differences must await further detailed surface analysis, it is likely that the performance differences of the two dentifrices are due to very different mechanisms of action. MI Paste contains casein phosphopeptide (RECALDENT™) that carries amorphous calcium phosphate. When the peptide complex binds to plaque or the tooth surface it is said to deliver bio-available calcium and phosphate for remineralization, resulting in occlusion of dentin tubules.

In comparison, the NovaMin®-containing SootheRx™ deposits fine particles onto the dentin or enamel surface and a series of reactions occurs over time that results in the formation of a crystalline, hydroxycarbonate apatite (HCA) layer. This HCA layer is chemically and structurally similar to natural enamel and dentin and is more resistant to acid challenges than the amorphous calcium phosphate deposited by the MI Paste.

The SEM and tubule occlusion data presented here clearly demonstrate the

difference in performance of the surface layers deposited by the two dentifrices. The MI Paste leaves a thin film on the structural dentin after 10 days, but there is very little occlusion of the dentin tubules. In comparison, the NovaMin® particles rapidly form a tenacious HCA layer on the dentin that resists the repeated acid challenges.

Conclusions

The current study has demonstrated that the NovaMin® particles are superior to the casein phosphopeptide (RECALDENT™) in depositing a mineral layer on dentin that will occlude dentinal tubules and resist demineralization by repeated acid challenges. This study supports the previous clinical studies that have demonstrated superior reductions in dentinal hypersensitivity in patients treated with NovaMin®-containing dentifrices compared with other commercially available products.