

Pilot clinical and *in vitro* studies evaluating NovaMin[®] in desensitizing dentifrices

NovaMin Research Report

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Abstract:

Objective: To evaluate bioactive glass (NovaMin[®]) as dentifrice ingredient capable of reducing tooth hypersensitivity through a mechanism of tubule occlusion.

Materials and Methods: A double-blinded, placebo-controlled clinical study with 66 adults was conducted with two test dentifrices containing 2.5% NovaMin and 7.5% NovaMin, respectively, formulated into an aqueous base. Cold air and tactile stimulation were used to evaluate sensitivity at baseline, 2, 4 and 8 weeks. Sensitivity was rated by participants on a visual analog scale (VAS) from 0-100mm. For inclusion in the study participants needed two teeth with sensitivity between 30-70mm on the VAS.

In vitro experiments were also performed to demonstrate the occlusion of open dentin tubules in a model designed to approximate the clinical study conditions. Experiments used standardized slabs of human dentin that had been prepared to exhibit open tubules through grinding and acid etching. Slabs were treated with the same dentifrices as were used in the clinical study, with an appropriate number of treatments to correspond with the Baseline 2, 4 and 8 week clinical study evaluations. Treatments consisted of passive application for 2 minutes, followed by 2 minutes of vigorous air/water rinse. The treated slabs were evaluated via Scanning Electron Microscopy (SEM) to look at tubule occlusion.

Results: In the clinical study both the Cold Air and Tactile measures showed significant reductions from baseline for test and control groups. The 7.5% formulation significantly outperformed both the control and the 2.5% formula at all time points. The 2.5% formula showed some improvement from control at early time periods, but lost this advantage at the 8-week time point.

Per Cent Reduction From Baseline

	Tactile VAS			Cold Air VAS		
	0%	2.5%*	7.5%*	0%	2.5%*	7.5%*
2 weeks	11%	28%*	43%*	5%	9%	26%*
4 weeks	29%	38%*	55%*	14%	28%*	43%*
8 weeks	35%	36%	73%*	28%	30%	55%*

*p<.05

The *in vitro* experiments demonstrated tubule occlusion as seen by visual examination under SEM, with apparent complete occlusion of all open tubules, while control dentifrice samples showed little or no occlusion. This appears to demonstrate that tubule occlusion is the mechanism of action for the clinical efficacy in sensitivity reduction.

Detailed report attached.

The Use of a Bioglass® Compound in a Dentifrice Formulation

Bioactive and biocompatible glasses have been developed as bone replacement materials. Studies have shown that these glasses will induce or aid osteogenesis in a physiologic system. Reaction of the glass begins immediately upon exposure to an aqueous environment. Sodium ions in the glass exchange with hydrogen ions from the body fluids causing the pH to increase. Calcium and Phosphorous migrate from the glass forming a Ca-P rich surface layer. This ion rich layer will interact with bone or tooth to form a bond and deliver chemical components to the tissue with positive effects. In bone the glass will bond and enhance the formation of new bone. In tooth structure, the glass will bond and release ions, change the pH and mechanically occlude open dentin tubules. This interaction with tooth structure can result in the reduction of tooth sensitivity, remineralization of tooth structure, whitening of tooth structure and potentially reduce the reattachment of calculus to tooth structure. Each of these effects will be described and supported by experimental data following.

INTRODUCTION

Tooth hypersensitivity is a common problem which affects about 40 million adults in the United States, 10 million of which can be considered chronically affected. It is estimated that some 17% of adults in the U.S. have at least one or more sensitive teeth. The teeth may be sensitive to cold, heat, air or sugary foods. The incidence of tooth hypersensitivity increases with age. The increased incidence is believed to be related to the general increase in exposed root surfaces of teeth as a result of periodontal disease, tooth brush abrasion or cyclic loading fatigue of the thin enamel near the dento-enamel junction.

The currently accepted theory for tooth hypersensitivity is called the hydrodynamic theory. This theory is based on the belief that open dentinal tubules allow fluid flow through the tubules. This flow excites the nerve endings in the dental pulp. Clinical replicas of sensitive teeth viewed in the scanning electron microscope (SEM) reveal varying numbers of open or partially occluded dentinal tubules. Tubules generally are not seen at the tooth root surface because of the cementum covering the tooth root, or because of a smear layer of dentinal debris 2-5 microns in thickness that covers the tooth surface and masks

the tubules. When the smear layer of the tooth is present, the fluid flow that can occur through the dentin is only a few percent of that possible following acid removal of the smear layer, thereby "opening" the tubules.

There is a growing body of evidence that occlusion of the dentinal tubules of a sensitive tooth, whether by resin infiltration, varnish coat or more recently by crystallite precipitation, results in reduction or elimination of the hypersensitivity. The duration of relief, however, is highly variable. Hypersensitivity usually reappears because of toothbrush abrasion, presence of acid challenges in the mouth or degradation of the coating material.

Desensitizing dentifrices containing potassium oxalate have been found to provide temporary tubule occlusion. Potassium oxalate is thought to react with the smear layer to increase its resistance to acid attack as well as reduce the permeability. It is thought that the crystals produced when dentin is treated with potassium oxalate are calcium oxalate.

Previously, all materials have used *biologically inactive* inorganic or organic components that will occlude the open tubules for a limited time period. Normal habits including the eating of acidic foods and vigorous toothbrushing will re-

move the materials from the tubules allowing fluid flow and a recurrence of sensitivity. In addition, literature has demonstrated with some hypersensitivity agents simple rinsing with water significantly reduces the number of occluded tubules. Therefore, there is a need in the dental field for a material that would chemically react with the surface of dentin and intimately bond to tooth structure, which would significantly reduce the reopening of dentin tubules due to contact with oral fluids and potentially remineralize the surface.

Bioactive and biocompatible glasses have been developed as bone replacement materials. Studies have shown that these glasses will induce or aid osteogenesis in a physiologic systems. The bond developed between the bone and the glass has been demonstrated to be extremely strong and stable. The bioactive glass formulation has been widely tested in bone and soft tissue. Toxicology evaluation of the glasses has shown no toxic effects in bone or soft tissue in numerous *in vitro* and *in vivo* models. However, the glass has been reported to be bacteriostatic or bacteriocidal most likely related to the change in pH induced by the dissolution of the ions from the surface of the glass and lack of bacterial adherence to the glass surface.

The bonding of the glass to bone begins with the exposure of the glass to aqueous solutions. Na⁺ in the glass exchanges with H⁺ from the body fluids causing the pH to become more alkaline. Ca and P migrate from the glass forming a Ca-P rich surface which will form an apatite layer. Underlying this Ca-P rich layer on the glass is an area which becomes increasingly silica rich due to the loss of Na, Ca and P ions.

Bioglass®, a bioactive glass, has not previously been described as a material for use in reducing tooth hypersensitivity. Subject of two patents (one allowed, one pending) is a novel Bioglass® compound which will mechanically occlude open tubules and release Ca and P to remineralize and strengthen tooth structure. In addition, sodium and calcium have been demonstrated to reduce transmission of nerve impulses, thus reducing the stimuli to the dental pulp.

Several studies have been completed to es-

tablish a proof of concept for this material. The following studies will be described: 1) Clinical trial using three concentrations of the Bioglass® compound in a dentifrice, 2) Scanning Electron Microscopy (SEM) studies to evaluate *in vitro* changes on dentin surfaces, 3) Fourier Transform Infrared Spectroscopy to identify apatite formation on dentin surfaces, and 4) SEM-Energy Dispersive Spectroscopy (SEM-EDS) on dentin surfaces to determine the presence of chemical components on the surface. All evaluations except the FTIR were performed using experimental dentifrice at the time intervals matching the examinations of the clinical trial.

MATERIALS AND METHODS

Clinical Study Design: The clinical trial was a "double-blind", placebo controlled study in which 66 healthy adults (Table 1.) with dentin hypersensitivity were enrolled. Sensitivity was confirmed at a screening appointment and subjects were randomly assigned to an eight week treatment schedule of unsupervised brushing twice a day with one of three dentifrices; a placebo(0%),

Participant Demographics				
	0%	2.5%	7.5%	Total
Number	22	22	22	66
Age	40.6	39.2	36.7	38.8
Male	4	4	4	12
Female	18	18	18	54
White	10	16	12	38
Black	11	5	10	26
Other	1	1	0	2

No significant differences among treatment groups using a Fisher's exact test

Table 1

2.5% Bioglass® compound and 7.5% Bioglass® compound (Tables 2 & 3). Efficacy measurements at Baseline, Two, Four and Eight Weeks of product use include cold-air and tactile sensitivity rated by the subject on a Visual Analog Scale (VAS) from 0-100 mm. An oral soft tissue examination and calculus evaluation were performed at each

Dentifrice Composition

Sorbitol 70% Solution
DI Water
Sodium Monofluorophosphate
Sodium Saccharin
Glycerin 96% USP
Carboxymethylcellulose
Sylodent 750
Sylodent 15
Peppermint Flavor
Sodium Lauryl Sulfate
Ethyl Alcohol 190 Proof
FD & C Blue #1

Table 2

visit to assess the safety of the product.

Study Population: Study participants were screened for eligibility based on a review of medical history and teeth responding to tactile and cold air stimuli. A full mouth examination was performed and two teeth identified for inclusion in

Dentifrice Composition Bioglass® Component

	0%	2.5%	7.5%
Sylodent 750	10%	7.5%	2.5%
Bioglass®	0%	2.5%	7.5%

Table 3

the study. A standardized stimulus using a Yeaple probe calibrated to deliver a 40 gram force perpendicular to tooth structure and a one second blast of cold air was applied. The subject rated the amount of sensitivity on a VAS and a score of 30-70 mm was necessary for inclusion of that tooth. Other inclusion/exclusion criteria are listed in Table 4.

Treatment: After meeting the inclusion/exclusion criteria, participants were randomly assigned to one of the three treatment groups. Subjects received a 120 gram tube of dentifrice and a soft bristle toothbrush at Baseline, 2 Week and 4 Week appointments. A supplemental tube was Bioglass® Dentifrice

available if necessary during the 4-8 week period. At each appointment, participants were evaluated for tactile sensitivity and cold air sensitivity and asked to rate it from 0 - 100 mm on the VAS scale. Patient compliance was measured by weighing the toothpaste tubes after collection and paste usage was monitored after completion of the study.

in vitro Design: Experiments were performed using a standardized slab of human tooth dentin from extracted teeth. These discs were cut from the extracted teeth using an Isomet diamond saw (Buehler, Ltd.). The discs were 1.0 mm thick and the size of the tooth. The occlusal surfaces were

Subject Inclusion/Exclusion Criteria

Subject inclusion criteria

- Aged 18-80 years
- Male or Female
- In good general health
- VAS between 30 - 70 mm at Baseline
- Minimum of 10 natural teeth
- No desensitizing toothpaste prior 30 days
- No dental prophylaxis during study

Subject exclusion criteria

- Dental pathology with similar symptoms
- Soft tissue pathology
- Orthodontic appliances
- FPD on subject teeth
- Chronic use of analgesic drugs
- Antibiotic prophylaxis
- Antibiotic usage greater than 7 days
- Allergy to any study products
- Excessive gingival inflammation
- Pregnant or lactating females

Table 4

ground on a series of wet silicon-carbide papers ranging from 320 to 600 grit. This was done to standardize the test surfaces. The surfaces were treated with 37% phosphoric acid for 60s to remove the smear layer created during the grinding process and open all dentin tubules (Figure 1.) The

surface was rinsed with distilled water for 20 s and dried with a blast of oil free air. Each slab was split in half and the experimental material placed on one-half of the specimen and the other half used as the etch control.

Scanning electron microscopy (SEM) was

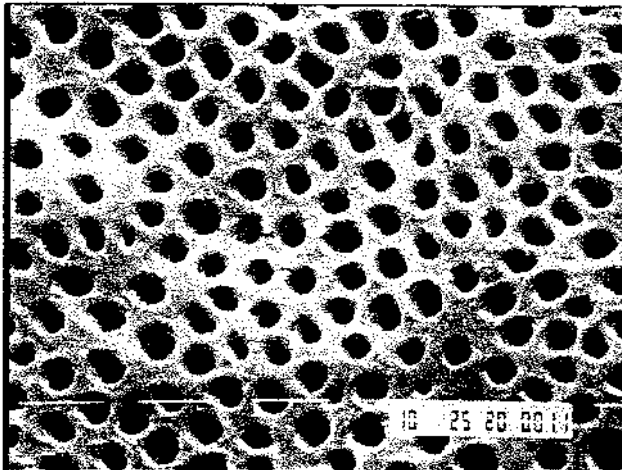


Figure 1. Acid Etched Control

performed on the treated surface in each group. The experimental side was treated for two minutes after etch by placing each dentifrice combination (0, 2.5, 7.5 %) passively two minutes followed by a thorough, vigorous air-water rinse for two minutes. Passive placement was used to eliminate potential smear layer formation. Specimens were prepared to match the time points corresponding to the clinical trial time points, Baseline, 2 Weeks, 4 Weeks and 8 Weeks. These samples would correlate with the sensitivity changes noted in the clinical trial. Twelve week samples were also prepared to evaluate aging of the paste preparation. The slabs were mounted on SEM stubs using silver paste. All specimens were vacuum dried, sputter coated with gold-palladium and examined in a JOEL-T200 scanning electron microscope. The control side was scanned for proper etch prior to evaluation of the treated side. Tubule penetration of the experimental material was evaluated by fracturing the treated side and evaluating the cross sectional view.

Scanning Electron Microscopy - Energy Dispersive Spectroscopy (SEM-EDS) was performed on samples prepared as above using a JEOL JXA-

840A Electron Probe Microanalyzer. The samples were sputter coated with gold-palladium which overlaps the energy spectra of P. Therefore, it was not possible to evaluate the concentration of P present on the surface.

Fourier Transform Infrared Spectroscopy (FTIR) was performed on samples treated as above without sputter coating. These analyses were used to identify hydroxyapatite peaks reforming on the surface of dentin blocks after disruption by acid etching.

RESULTS AND DISCUSSION

Mean changes in VAS scores are reported in Tables 5-7. Mean changes from Baseline, Cold Air VAS (Table 5, Figure 2) show a significant reduction over the eight week period. The 7.5% was significantly different from the 0% composition at all time points. In addition, there was a cumulative reduction in cold air sensitivity over the eight weeks.

Mean changes from Baseline, Tactile VAS

Mean Changes from Baseline Cold Air VAS				
Time	0%	2.5%	7.5%	p-value
2 weeks	-2.6	-5.1	-13.1	0.044
4 weeks	-6.8	-14.5	-21.5	0.024
8 weeks	-14.5	-15.5	-27.4	0.044

Table 5

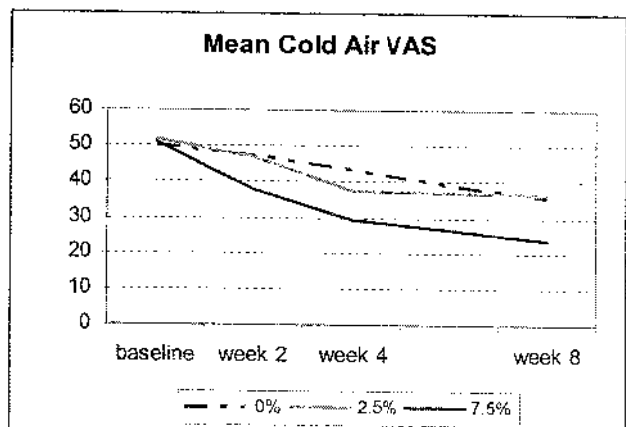


Figure 2

(Table 6, Figure 3.) show a significant reduction over the eight week period. The 7.5% composition was significantly different from the 0% composition at all time points. In addition, there was a cumulative reduction in tactile sensitivity over the eight weeks.

Mean (% Reduction) Cold Air VAS (Table

Mean Changes from Baseline Tactile VAS				
Time	0%	2.5%	7.5%	p-value
2 weeks	-5.1	-13.6	-21.1	0.016
4 weeks	-13.7	-18.6	-27.5	0.049
8 weeks	-16.5	-17.6	-36.0	0.001

Table 6

7.) shows a significant reduction in cold air sensitivity over the study period. In addition, a trend

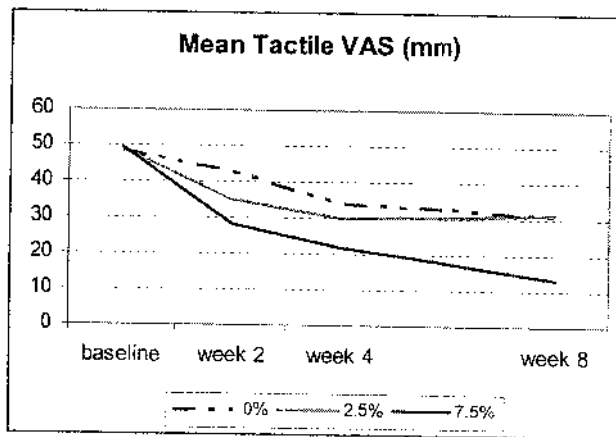


Figure 3

can be noted relative to the rate and concentrations of the compositions. At the two week examination the 7.5% already shows a 26% reduction in sensitivity to cold air. At four weeks the 7.5% shows a 43% reduction and the 2.5% shows a 28% reduction which is significantly different than the 0%. This trend continues with the 7.5% resulting in a continued reduction of 55% through the eight week period. From these data a dose dependent effect of the Bioglass® compound is seen.

The reduction of sensitivity to cold air shows both a more rapid response and a greater magnitude of reduction with the higher concentrations of

Mean (% Reduction) Cold Air VAS				
Time	0%	2.5%	7.5%	ANOVA
Base	48.9	50.0	49.5	NS
2 wk	46.4 (5%)	45.1 (9%)	36.7 (26%)	S
4 wk	42.3 (14%)	35.8 (28%)	28.4 (43%)	S
8 wk	35.1 (28%)	34.8 (30%)	22.3 (55%)	S

Table 7

Bioglass® in the test dentifrice.

Mean (%Reduction) Tactile VAS (Table 8.) shows a significant reduction in tactile sensitivity over the study period. The trend noted above for the cold air is even more pronounced with the reduction in tactile sensitivity. At the two week time point the 7.5% already shows a 43% reduction with the 2.5% showing a 28% reduction in sensitivity. The amount of reduction in sensitivity continues with the resultant decrease in tactile sensitivity reaching 73% at the eight week time point

Mean (% Reduction) Tactile VAS				
Time	0%	2.5%	7.5%	ANOVA
Base	48.5	48.9	49.4	NS
2 wk	42.8 (11%)	35.2 (28%)	28.4 (43%)	S
4 wk	34.1 (29%)	30.0 (38%)	22.0 (55%)	S
8 wk	31.1 (35%)	31.2 (36%)	13.4 (73%)	S

Table 8

for the 7.5% composition. Again the dose dependent response is evident over the eight week period with the higher concentrations of the

Bioglass® compositions showing a more rapid and greater magnitude of reduction in tactile sensitivity.

Scanning Electron Microscopy - Energy Dispersive Spectrum analysis (Figure 4) shows a high concentration of calcium and the presence of silica and sodium on the surface analysis of a four week dentin block sample treated with the 7.5% dentifrice composition. The presence of the high levels of calcium can only be coming from the Bioglass® component of the dentifrice since there is no other source present. This further supports the conclusion that the Bioglass® compound is supplying chemical components for remineralization of the dentin surface.

Representative Scanning Electron Micrographs are presented. The acid etch control (Figure 5) is a view of the dentin surface after etching with 37% phosphoric acid. Note the absence of smear layer debris and presence of wide open tubules that are also absent any debris. This surface was the starting point for all SEM's. The placebo (Figure 6) shows the application of the dentifrice with 0% Bioglass®. Note the absence of any tubule occlusion and minimal debris on the dentin surface. Two SEM's are presented after application of the 7.5% Bioglass® dentifrice (Figures 7 & 8). Note the almost total occlusion of the tubules and the beginning of formation of hydroxyapatite crystals.

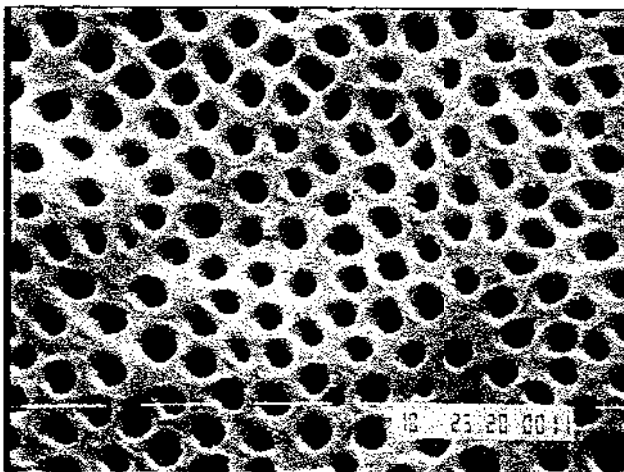


Figure 5. Acid Etch Control 1,5000x

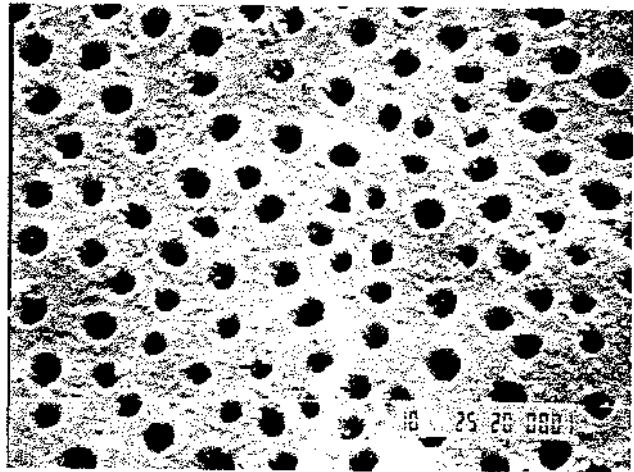


Figure 6. 0% Bioglass® Dentifrice 1,500x

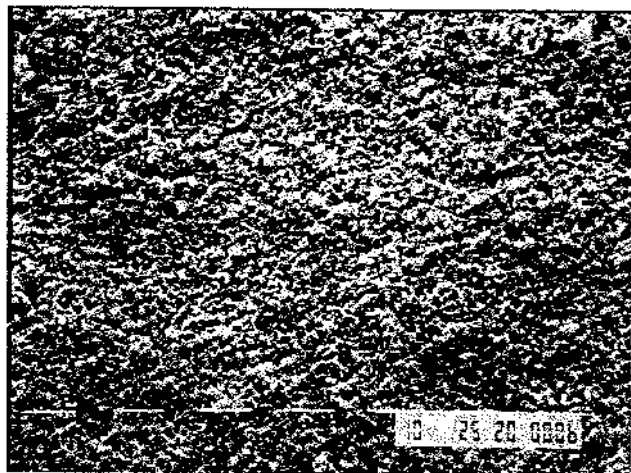


Figure 7. 7.5% Bioglass® Dentifrice 1,000x

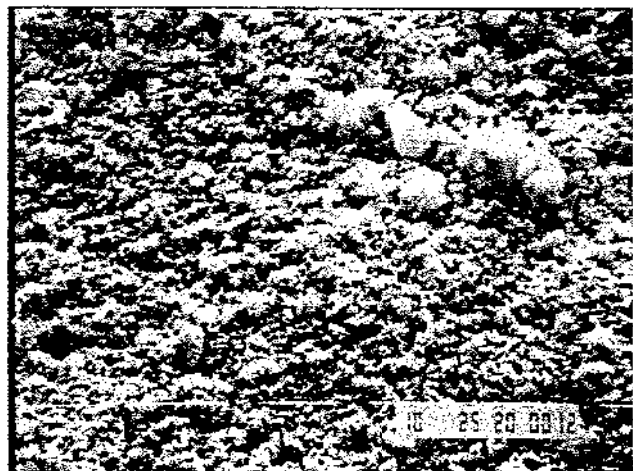


Figure 8. 7.5% Bioglass® Dentifrice 1,500x

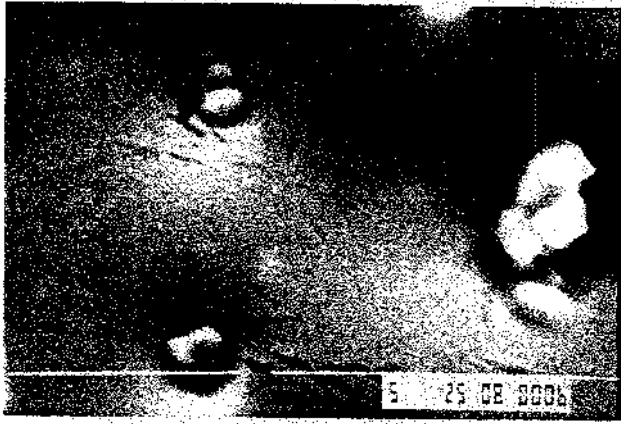


Figure 9 Bioglass® Varnish

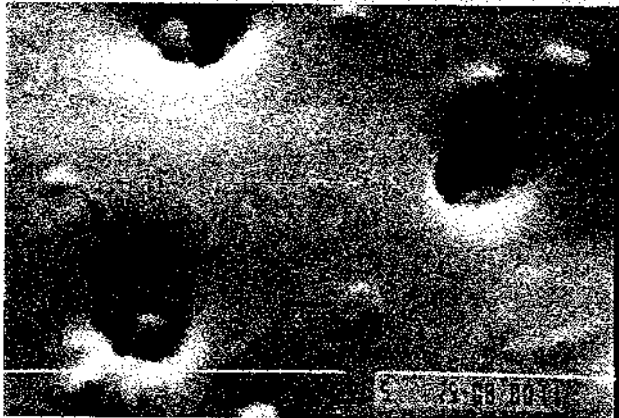


Figure 10. Bioglass® Varnish

Varnish: Representative SEM's of dentin block samples are presented in Figures 9 & 10. Particulate Bioglass® was added to a cavity varnish and placed on etched dentin blocks. Note the particulate glass both under the varnish and penetrating the tubules with the dissolution of the particles, the release of Ca and P ions will allow a remineralization of the surface as well as occlusion of dentin tubules. This would effectively block any hydrodynamic changes causing tooth sensitivity. The varnish development is ongoing and research projects related to cavity liners and use under crowns are currently being performed.

CONCLUSIONS

- 1) The Bioglass® dentifrice significantly reduced dentin hypersensitivity in a controlled eight week efficacy clinical trial.
- 2) Clinical data show the appearance of dose related effects, that is, a higher concentration resulted in a more rapid and greater magnitude of sensitivity reduction.
- 3) Laboratory data using the Bioglass® dentifrice on SEM, SEM-EDS and FTIR confirm the presence of the Bioglass® compound on the dentin surface.
- 4) Laboratory data show the presence of sodium and calcium on the treated surfaces coming from the Bioglass® which is the only reservoir of these components.
- 5) Mechanisms of sensitivity reduction include:
 - a) Rapid mechanical occlusion of tubules with penetration.
 - b) Chemical bond to the dentin surface.
 - c) Release of sodium and calcium with a decrease in the rate of nerve impulse transmission.
 - d) Release of calcium and phosphorous for remineralization which produces long term sensitivity reduction.