IN-VITRO EVALUATION OF NOVAMIN® ROOT CONDITIONER

1. BACKGROUND & PRECEDENCE

Tooth hypersensitivity is a common problem that affects approximately 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.\(^1\) Tooth hypersensitivity increases with age, and is attributed to the general increase in exposed root surfaces of the teeth from periodontal disease, toothbrush abrasion, or cyclic loading fatigue of the thin enamel near the Cemento-Enamel Junction (CEJ).

The currently accepted theory for tooth hypersensitivity is the hydrodynamic theory proposed by Brännström.\(^4\) This theory is based on the belief that open dentinal tubules allow fluid flow through the tubules, which excites the nerve endings in the dental pulp. Clinical replicas of sensitive teeth viewed under a Scanning Electron Microscope (SEM) reveal varying numbers of open or partially occluded dentinal tubules.\(^6\),\(^7\),\(^8\) In general, tubules 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 thick that covers the tooth surface and masks the tubules. When the smear layer is present, the fluid flow that can occur through the dentin is only a percent of that possible following acid removal of the smear layer, which “opens” the tubules.

There have been two basic approaches to the treatment and prevention of dentinal hypersensitivity. The first approach is to treat the tooth with a chemical agent that penetrates into the dentinal tubules and depolarizes the nerve synapse, which reduces sensitivity by preventing the conduction of pain impulses (e.g., potassium nitrate).\(^9\),\(^10\) The second approach is to treat the tooth with a chemical or physical agent that creates a deposition layer and mechanically occludes dentinal tubules, which reduces sensitivity by prevention of pulpal fluid flow (e.g., potassium oxalate, ferric oxalate).\(^11\),\(^12\) Although both approaches are effective at reducing or eliminating hypersensitivity, the duration of relief is highly variable. Hypersensitivity usually reappears due to toothbrush abrasion, presence of acid challenges in the mouth, and/or degradation of the coating material.\(^13\),\(^14\),\(^15\),\(^16\),\(^17\),\(^18\),\(^19\),\(^20\)

Progress has been made towards meeting this need through the development of products that deposit calcium phosphate (Ca-P), a mineral that is chemically similar to natural dentin, onto the tooth surface to mechanically occlude dentinal tubules. One commercially available product, Quell™ Desensitizer, uses this approach. Quell™ Desensitizer is supplied in two phases: one containing an aqueous solution of calcium chloride (CaCl₂) and the other containing an aqueous solution of potassium phosphate (K₃PO₄). The first phase is applied to the surface of the tooth and is immediately followed by application of the second phase over the first. This mixing action results in the precipitation of calcium phosphate (Ca-P), which occludes dentinal tubules and provides relief from hypersensitivity.

The performance studies summarized herein utilize a new material, NovaMin®, which can physically occlude dentinal tubules. NovaMin® is a trade name that has been given to bioactive glass (e.g., Bioglass®) that has been ground into a fine particulate with a median size of less than 20 microns. NovaMin® has been shown in vitro and in vivo (US Patents 5,735,942 and 6,086,374)\(^21\),\(^22\) to reduce sensitivity by blocking open tubules and by supplying calcium (Ca²⁺) and phosphate (PO₄³⁻) ions in an optimum environment to form hydroxyapatite (HCA).

The physical occlusion of NovaMin® particles begins when the material is subjected to an aqueous environment. Sodium ions (Na⁺) in the particles immediately begin to exchange with hydrogen cations (H⁺ or H₃O⁺).\(^23\) This rapid release of ions allows calcium (Ca²⁺) ions in the particle structure, as well as phosphate (PO₄³⁻) ions to be released (Ca-P) layer. As the particle reactions
continue and the deposition of calcium phosphate complexes continue, this layer crystallizes into a calcium hydroxyapatite, also known as hydroxycarbonate apatite, that is chemically and structurally equivalent to biological apatite. The combination of the residual NovaMin® particles and the hydroxycarbonate apatite layer results in the physical occlusion of dentinal tubules, which will relieve hypersensitivity.

Clinical trials performed by Litkowski, et al showed efficacy of a Bioglass®-containing dentifrice (2.5 and 7.0% w/w) in significantly reducing patient perceived pain to stimuli with daily use. These trials were performed over an eight-week period with follow up interviews up to twelve weeks after cessation of product use. These data indicate both a significant reduction and a long-lasting reduction in hypersensitivity.

The studies summarized herein utilized a new root conditioner device that is approximately 60% w/w NovaMin® when mixed with the supplied aqueous carrier. Upon mixing, a thick flowable paste is formed, which can be applied directly to the tooth surface. This new device will be applied by a clinician to either dentin exposed by gingival recession or to dentin exposed during periodontal procedures. (In either case, the exposed dentinal tubules are the source of the hypersensitivity.)

The studies summarized herein provide documentation of efficacy. The primary purposes of the studies were to document the degree of tubule occlusion produced by a single application of a NovaMin®-containing root conditioner product as compared with positive and negative controls and to determine the length of time that the tubules remain occluded.

2. VALIDATION OF TUBULE OCCLUSION EFFICACY IN VITRO

a) Purpose. An in vitro dentin block model was used to assess the tubule occlusion performance of NovaMin® Root Conditioner relative to both untreated controls and the predicate device, Quell™ Desensitizer. In addition, the in vitro study assessed the material characteristics of NovaMin® Root Conditioner and the predicate device, Quell™ Desensitizer, to demonstrate substantial equivalence. This in vitro study used prepared dentin samples to guarantee tubule exposure to facilitate statistical evaluation of the data collected.

b) Method. Extracted bovine incisors were used for the in vitro study. The teeth were initially scraped to remove all soft tissue, and then the roots were cut off at the cemento-enamel junction (CEJ). Dentin blocks were prepared from the roots by grinding a flat spot on the buccal surface through the cementum layer. The root dentin blocks were then sonicated three times to remove grinding and polishing debris. Following sonication, the root dentin blocks were etched using 37% phosphoric acid (H₃PO₄) for 15 minutes to remove any smear layer from the grinding process. Following etching, the root dentin blocks were again sonicated three times to thoroughly clean. The root dentin blocks were stored in Tris buffer and under refrigeration prior to use.

The test specimens were separated into three treatment groups and treated accordingly:

- Negative Control – no additional treatment
- Test Group – treated with NovaMin® Root Conditioner
- Positive Control – treated with the predicate device, Quell™ Desensitizer

Following treatment, the specimens were dried and prepared for analysis by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). Once the SEM images had been obtained, the numbers of fully-open and partially-open tubules present on the surface of each sample were manually counted. The tubule counts were then used to provide a statistical basis for evaluation. The EDS spectra were used to qualitatively evaluate the chemical structure of the surface of the samples and to validate the presence of calcium phosphate.

c) Results. The results of the evaluation show that NovaMin® Root Conditioner is effective at occluding dentin tubules. The SEM images shown in Figures 1a, 1b, and 1c demonstrate the tubule occlusion efficacy of NovaMin® Root Conditioner relative to the positive and negative controls.
The number of tubules evident in each of the 2000X images collected were counted to provide a measure of tubule occlusion efficacy. Both the fully-open tubules and the partially-open tubules were included in the count to be conservative (i.e., this method assumes that if a tubule is only partially occluded, then it does not block enough hydrodynamic flow to affect desensitization). Descriptive statistics were then generated for each group based upon the tubule count data collected and are summarized in Table I.

### Table I. Descriptive Statistics for Tubule Count Data

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Number of Samples</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control (untreated)</td>
<td>24</td>
<td>37.79</td>
<td>20.58</td>
<td>4.20</td>
</tr>
<tr>
<td>TEST (NovaMin®)</td>
<td>25</td>
<td>6.96</td>
<td>11.01</td>
<td>2.20</td>
</tr>
<tr>
<td>Positive Control (Quell™)</td>
<td>25</td>
<td>26.00</td>
<td>21.65</td>
<td>4.33</td>
</tr>
</tbody>
</table>

A univariate analysis of variance (ANOVA) performed on the tubule counts showed significant differences between the means, $F(2,71) = 17.669$, $MSE = 336.661$, $p < .001$. Bonferroni post hoc tests revealed significantly fewer tubules in the NovaMin® treatment condition relative to both the positive control ($p < .001$) and the negative control ($p < .001$). The results of the post hoc tests are summarized in Table II.

### Table II. Bonferroni Post Hoc Tests for Tubule Count Data

<table>
<thead>
<tr>
<th>Conditions Compared</th>
<th>Mean Difference</th>
<th>Standard Error</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control (untreated) s. TEST (NovaMin®)</td>
<td>-30.83*</td>
<td>5.24</td>
<td>$p &lt; .001*$</td>
</tr>
<tr>
<td>Positive Control (Quell™) s. TEST (NovaMin®)</td>
<td>-19.04*</td>
<td>5.19</td>
<td>$p &lt; .001*$</td>
</tr>
<tr>
<td>Negative Control (untreated) s. Positive Control (Quell™)</td>
<td>-11.79</td>
<td>5.24</td>
<td>$p &lt; .081$</td>
</tr>
</tbody>
</table>

* Denotes significant difference at the $\alpha=.05$ level

In addition to SEM images, EDS spectra were collected on each sample to examine the elements present on the tooth surface. The EDS spectra shown in Figures 2a, 2b, and 2c demonstrate the formation of a calcium phosphate layer (i.e., hydroxycarbonate apatite) by the reaction of NovaMin® particles and its similarity to the calcium phosphate layer deposited by the predicate device, Quell™ Desensitizer. The calcium phosphate layer can be readily identified by the large calcium (Ca) and phosphorous (P) peaks in each spectra. The samples containing NovaMin® are easily identified by the presence of a silicon (Si) peak, which is the result of the amorphous silica present in the material structure.
d) **Discussion.** The *in vitro* study summarized above demonstrates that NovaMin® Root Conditioner occludes a significant number of dentinal tubules relative to untreated controls ($p < .001$). In addition, the study shows that under the conditions of the test NovaMin® Root Conditioner occludes significantly more tubules than the predicate device, Quell™ Desensitizer ($p < .001$). Finally, the study demonstrates that the calcium phosphate layer produced by NovaMin® is elementally similar to the calcium phosphate layer produced by Quell™ Desensitizer, which substantiates equivalence between the two materials.

3. **VALIDATION OF CALCIUM PHOSPHATE LAYER PERSISTENCE IN VITRO**

a) **Purpose.** An *in vitro* immersion model was used to validate the persistence of the calcium phosphate layer produced by NovaMin® Root Conditioner. The test was designed to simulate immersion of the device in an oral environment following application. This *in vitro* study used unprepared dentin samples to simulate clinical application of the device.

b) **Method.** The *in vitro* study was performed using extracted bovine incisors. The teeth were initially cleaned by removing all soft tissue, and then the roots were cut off at the cemento-enamel junction (CEJ). The tooth root specimens were then etched with EDTA (ethylenediaminetetraacetic acid, 0.5M concentration, pH = 7.4) for 4 minutes to remove any smear layer. The tooth root specimens were separated into three groups and treated accordingly:

- **Negative Control** – no additional treatment
- **Test Group** – treated with NovaMin® Root Conditioner
- **Positive Control** – treated with the predicate device, Quell™ Desensitizer

One set of samples from each group were retained to reflect the “as treated” condition at baseline. A second set of samples from each group was immersed for 7 days at 37°C in a neutral pH simulated saliva solution consisting of approximately 66.6% w/w gastric mucin, 9.9% w/w sodium phosphate dibasic heptahydrate, 9.9% w/w calcium chloride, 6.7% potassium chloride, 6.7% sodium chloride, 0.1% w/w sodium sulfide nonahydrate, and 0.1% w/w magnesium pyrophosphate.

Following the soaking period, the specimens were dried and prepared for analysis by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). The SEM images were used to qualitatively evaluate the persistence of the calcium phosphate layer produced by NovaMin® Root Conditioner by examining the extent of tubule occlusion relative to the positive and negative controls. The EDS spectra were used to qualitatively evaluate the chemical structure of the surface of the samples and to validate the presence of calcium phosphate.

c) **Results.** The SEM images shown in Figures 5a, 5b, and 5c confirm the tubule occlusion resulting from calcium phosphate deposition by NovaMin® Root Conditioner at baseline. The SEM images shown in Figures 5d, 5e, and 5f demonstrate the persistence of the calcium phosphate layer and tubule occlusion following a 7-day immersion in a simulated oral environment.
The EDS spectra shown in Figures 6a, 6b, 6c, 6d, 6e, and 6f confirm the formation of a calcium phosphate layer (i.e., hydroxyapatite) by the reaction of the NovaMin® particulate and its similarity to the structure of both the positive and negative controls. The calcium phosphate layer can be identified by the calcium (Ca) and phosphorous (P) peaks, and NovaMin® can be identified by the presence of a silicon (Si) peak. Note that the silicon (Si) in the NovaMin®-containing samples significantly reduced between baseline and 7-days, indicating the reaction of the bulk of the NovaMin® particles to form hydroxyapatite. Also note the similarity between the calcium phosphate layer produced by both NovaMin® and Quell™ relative to natural dentin.
d) **Discussion.** The data shows that NovaMin® Root Conditioner forms a protective calcium phosphate layer (i.e., hydroxycarbonate apatite) that is sufficient to occlude exposed dentinal tubules for a period of at least 7-days when immersed in a simulated oral environment. Qualitative evaluations indicate that the tubule occlusion achieved by NovaMin® Root Conditioner is approximately equal to the predicate device, Quell™ Desensitizer.

4. **REFERENCES**


