ABSTRACT

Introduction: Dentin hypersensitivity is a widespread issue with high prevalence. Blocking the open dentinal tubules is one way to reduce dentin hypersensitivity.

Aim: The current study aimed at evaluating the in vitro potential of a novel experimental dentifrice made of nano-fluoro hydroxyapatite (n-FHAP) produced from a biogenic source (cuttlefishbones) for occlusion of dentinal tubules.

Material and Methods: 30 dentin discs (n = 30) were classified into 3 categories (n = 10) based on the dentifrices they utilized for brushing: 1st category = artificial saliva (AS; control); 2nd category = fluoride dentifrice (Sensodyne, UK); 3rd category = experimental nano-FHAP dentifrice. Then, after brushing, discs were subjected to an acidic challenge with 6 per cent wt citric acid (pH = 4.0) for 1 minute. By utilizing a SEM (scanning electron microscope) and EDX (energy-dispersive X-ray) spectroscopy before and after the citric acid challenge, the tubule occlusion was measured.

Results: Group 1 (AS) SE micrographs revealed no tubule blockage. The tubules in Groups 2 and 3 were mostly blocked, according to SE micrographs. Data before and after the citric acid challenge data were statistically significant (p= 0.05) for all groups.

Conclusion: In terms of tubule blockage, the findings of the new experimental dentifrice were equivalent to those of Sensodyne. Dentifrice containing FHAP nano-particles might be used to treat dentin sensitivity as an alternative.

INTRODUCTION

Described as a mineral-bearing tissue, Dentin hypersensitivity (DH) is comprised of small dentinal tubules exposed because of enamel loss and gingival recession (1). DH is a widespread issue, with prevalence estimates as high as 74% (1). The hydrodynamic theory states that the exposure of dentinal tubules generates the hypersensitivity, where the nerve endings in the pulp are stimulated by the flow of fluids as a response to stimuli, and intense pain is generated for a brief time (2). The extrinsic variables contributing to tooth erosion and tubule exposure are associated with dietary or medicinal acids, as well as specific behavioural factors. The reasons of DH include fractures, caries, tooth wear, and gingival recession (3). Dentin may become sclerotic as the tubules get blocked by minerals as people age (4). However, as this is a lengthy procedure, patients may stay in agony for longer period.
As been previously indicated, the use of desensitizing dentifrices can alleviate DH in a quick and efficient manner (5). Demineralization can be reversed as well by applying mineralizing chemicals to the tooth surface. A form of mineralizing agent that is capable of chemically attaching to hard tooth tissues is fluoro-hydroxyapatite (FHAP) (6). These hydroxyapatites were initially developed for osteogenesis; nevertheless, their use in different dental products, particularly dentifrices, has increased since then (7). Because the structure of bone and dentin is similar, using fluoro-hydroxyapatites on dentin might help achieve therapeutic advantages (8). In order to ensure the development of robust fluorapatite (FAP) crystals, additional fluoride would be advantageous (9).

One of the commercially available toothpastes is Synsodyne, which has shown strong characteristics of tubule occlusion characteristics in various in vitro studies (10). Kanwal et al. (11) found that when fluoride is included into a dental paste, it contributes to fluoridated apatite generation in Tris buffer solution that has superior clinical durability, as shown by a recent in vitro study. Furthermore, fluoride in toothpaste combined with other desensitizing compounds has shown to be more effective in caries prevention and desensitization (12).

Up to sum, the goal of this study was to create n-FHAP from a biologic source, test them for dentin tubule occlusion, and compare the findings to those of a conventional fluoride dentifrice (Synsodyne). All studied dentifrices will have equal dentin tubule occlusion capability, according to the null hypothesis (H0).

### MATERIAL AND METHODS

1. **Dentifrices Composition:**

The dentifrice composition utilized in this investigation is shown in table no.1.

<table>
<thead>
<tr>
<th>Dentifrice</th>
<th>Composition</th>
<th>Active ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synsodyne</td>
<td>Potassium Nitrate, Sodium Fluoride, Potassium Nitrate, Sodium Mono-fluorophosphate, Baking Soda, Tartar Control.</td>
<td>Sodium Fluoride</td>
</tr>
<tr>
<td>Experimental</td>
<td>n-FHAP powder, Sodium benzoate 1%,glycerine, Methylcellulose, Sodium Lauryl sulphate</td>
<td>FHAP nanoparticles</td>
</tr>
</tbody>
</table>

2. **Dentin Disc Preparation and Specimen Grouping:**

Non-curious teeth were obtained from the Faculty of Dentistry, Suez Canal University’s Oral and Maxillofacial Surgery Clinics for this investigation. By utilizing a cooled diamond saw (Isomet® 5000 Linear Precision Saw, Buehler Ltd., Lake Bluff, IL, USA), each tooth was cut horizontally (mesio-distally) across cemento-enamel junction yielding thirty 2.0 mm (0.2 mm) dentin discs. Then slicing teeth mesio-distally, 2.5 mm below the cusp tip of the occlusal enamel was removed as well. Using 37 percent orthophosphoric acid for 20 seconds, then the dentin discs’ upper surfaces were etched in order to open the tubules and liberate them of any organic material. After etching and before separating the discs randomly in 3 groups, 10 discs each category (n=10), then the discs were rinsed in distilled water for a minute. Then, they were treated with different dentifrices as follows: discs in Group 1 were brushed using artificial saliva (AS;control), discs in Group 2 were brushed using fluoride dentifrice (Synsodyne, UK); and discs in Group 3 were brushed using the experimental n-FHAP agent dentifrice. According to Fusayama et al. (13), the AS was prepared via the combination of the following ingredients in 1000 mL of deionized water: H₂O: 0.69 g; CaCl₂ H₂O: 0.795 g; Na₂S. 9H₂O: 0.005 g; NaCl: 0.400 g; KCl:
0.400 g; NaH₂PO₄: 0.400 g. The freshly produced Artificial saliva AS had a pH of 5.5, which was adjusted to 7.0 by adding NaOH[^10].

3. Biogenic source cuttlefish (sepia officinalis) bones (CB) are used to synthesize FHAP nano-particles:

Wet mechano-synthesis and subsequent oven drying were used to make hydroxyapatite from CB powder.

The powder was first combined with ammonium fluoride as a fluoride source, then with ammonium phosphate dibasic (NH₄)₂HPO₄ to produce a Ca/P ratio of 1.67. Ball-milling the reactants in an aqueous solution with a Turbula® mixer aided the mechano-synthesis reaction. The resultant slurry was dried for 24 hours in an oven[^14].

4. Formulation of a dentifrice out of the synthesized n-FHAP:

The n-FHAP powder was combined with distilled water to produce slurry. Then, to make a dentifrice, it’s mixed with Methylcellulose. Glycerine was added to improve the workability of the product. As a preservative, sodium benzoate was used. As a foaming agent, sodium Lauryl sulphate was used.

5- Tooth Brushing Protocol for Dentin Discs:

For 7 days, discs in the 1st group were brushed with 1 mL AS and discs in the other groups were brushed using 1 g of their respective dentifrices two times a day (one time in the morning and one time in the afternoon). Combining the dentifrice in a 1:2 ratio with deionized water, a load of 200 g was applied to the head of the toothbrush (Medium bristled, Oral B®, Pro-Flex TM, USA) for ensuring that samples were touched. Each day after brushing, discs were rinsed in distilled water for a minute, then they were returned to their individual containers that contain AS at 37°C.

6. Citric Acid Challenge:

Discs were subjected to a citric acid (6 percent wt.) challenge for a minute after brushing. To make citric acid fresh, 6 g of citric acid powder (Fun Fresh Foods, San Clemente, CA, USA) was combined with 100 mL deionized water and the pH was kept at 4. Then, after exposure to citric acid, discs were rinsed with distilled water and allowed to air dry for 24 hours before being analyzing them using SEM (scanning electron microscope).

7. Energy-Dispersive X-Ray Analysis (EDXA) of Tubule Occlusion:

After placing the dentin discs on stubs, they were sprayed with gold. Afterwards, they were examined with a SEM (FEI, Inspect F50, The Netherlands) equipped with energy-dispersive spectroscopy (EDS, Oxford Instruments, UK). Then, they were tested before and after being exposed to citric acid. 5 and 20 kV electron modes were used at 10 and 15 Kx magnifications from the center locations highlighted on the discs to obtain the micrographs. Pre and post acid challenge, the Ca/P ratio was determined for all groups.

8. Statistical Analysis:

For data analysis, SPSS-20.0 was utilized (IBM product, Chicago, IL, USA) to evaluate the levels of pre- and post- citric acid challenge tubule occlusion within each group. A paired sample T-test was employed to evaluate pre- and post-citric acid challenge tubule occlusion levels. For comparing the findings of groups’ post citric acid challenge, Tukey’s post hoc test was conducted. The difference among means was found to be statistically significant and was defined as a p value of less than 0.05.
RESULTS

For pre- and post-citric acid challenges, SEM micrographs of the 1st group (AS) did not show any tubule blockage (0 percent). While, the SEM micrographs of the 2nd and 3rd groups exhibited superior tubular occlusion, with most tubules remaining occluded, as shown in figure 1a, b, and c.

After the citric acid challenge, the dentin surface’s elemental composition was determined using energy-dispersive X-rays in all groups. For all groups, the paired sample t-test revealed that the changes (p=0.05) between the values of pre- and post-citric acid challenge were statistically significant (excluding group 1). As demonstrated in Table no.2, after the citric acid challenge, the intergroup comparisons revealed statistically significant variations (p 0.05) in the levels of tubule occlusion.

![Fig. (1): a: Group 1 (AS), b: Group 2 (Sensodyne), and c: Group 3 (Experimental)](image)

| Table (2) | Pairwise comparison of elements atomic % of different groups in Dentin: |
| --- | --- | --- | --- | --- | --- |
| **Element** | **Groups** | **Atomic %** | **p-value adjusted for Pairwise-Comparisons** |
| | | **Mean** | **SD** | **Artificial Saliva** | **Sensodyne©** |
| Calcium | Artificial Saliva | 15.08 | 0.90 |  |  |
| | Sensodyne© | 22.98 | 1.50 | <0.001* |  |
| | n-FHAP dentifrice | 19.43 | 0.87 | 0.033* | 0.032* |
| Phosphorus | Artificial Saliva | 11.65 | 0.57 |  |  |
| | Sensodyne© | 13.32 | 0.80 | <0.001* |  |
| | n-FHAP dentifrice | 12.81 | 0.61 | 0.018* | 0.856 |
| Carbon | Artificial Saliva | 16.19 | 1.17 |  |  |
| | Sensodyne© | 10.05 | 2.88 | <0.001* |  |
| | n-FHAP dentifrice | 17.04 | 7.04 | 0.465 | 0.015* |
| Ca/P ratio | Artificial Saliva | 1.30 | 0.10 |  |  |
| | Sensodyne© | 1.73 | 0.16 | <0.001* |  |
| | n-FHAP dentifrice | 1.52 | 0.09 | 0.033* | 0.062 |

* Statistically significant p-value
DISCUSSION

The tubule occlusion competence of a new n-FHAP experimental dentifrice was studied, and its competence was compared to Synsodyne and AS. Tubule occlusion was not observed in the AS-containing control group. Human saliva has a crucial role to play in delivering essential ions including calcium and phosphate to the dentin surface. However, such mechanism alone is insufficient to occlude the tubules and reduce DH (11). Because of the significant link between fluoride usage and the prevention of dental caries (15), fluoride incorporation in dentifrices is advantageous to tooth structure. Fluoride has a contribution of FAP production from HAP by substituting the hydroxyl ion in the apatite structure of dentin for the fluoride ion (16). The results show that Synsodyne dentifrice was able to block the majority of tubules (>50 and 100%) both pre- and post- citric acid challenge. Synsodyne shows superior blocking of dentin disc’s patent tubules than Novamin dentifrice, according to Arnold et al. (17). Synsodyne showed comparable tubule occlusion competence in the current research. In comparison to Synsodyne, our new n-FHAP-containing dentifrice produced similar effects. As its surface interacts with dentin that confirms increased open tubules’ plugging, including n-FHAP particles in such dentifrices may have resulted in better tubule occlusion. When presented with an acidic challenge, the best dentifrice for the treatment of DH should not only block tubules but maintain tubule occlusion as well (10). This study’s experimental dentifrice occluded the majority of tubules while also retaining the majority of tubule occlusion. The inclusion of fluoride in the experimental dentifrice formulation provides a gradual and prolonged fluoride release, with expecting the long-lasting development of FAP instead of fluorite (6). The in vitro aspect of this work may restrict the ability to acquire entirely accurate data, as in vivo circumstances may differ significantly from in vitro settings. For having more understanding of the influence of these experimental dentifrices in genuine dynamic in-vivo settings, well-controlled clinical studies are needed. The conclusions of this study should only be regarded as preliminary findings that should be followed up with a long run assessment of the examined dentifrices.

CONCLUSION

The experimental n-FHAP dentifrice was shown to be equally able to occlude dental tubules as the fluoride dentifrice, with similar outcomes. The findings may help dentists make appropriate dentifrice recommendations for patients, particularly those with dentin sensitivity.

REFERENCES


