The effect of nano-hydroxyapatite toothpaste on enamel surface remineralization. An in vitro study

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The effect of nano-hydroxyapatite toothpaste on enamel surface remineralization. An in vitro study

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ABSTRACT: Purpose: To compare, in vitro, the surface remineralization effect of two fluoride dentifrices. **Methods:** 90 human enamel specimens, embedded in cylindrical acrylic blocks, washed and polished, served as a study material. The specimens were randomly assigned in equal numbers to the three groups (two test and one control). Initially the microhardness of the enamel surface (SMH) was assessed by Vickers method, while a profilometer was used to evaluate the surface roughness (Ra value). The enamel specimens were subjected to the preliminary demineralization, simulating the initial carious lesion formation. Subsequently, mean SMH and Ra values were re-assessed. Upon demineralization, one half of each specimen was covered with a protective varnish. The exposed fragment underwent the 3-week cycle of pH changes, according to the protocol. The groups were as follows: NHAPF group – toothpaste containing nanohydroxyapatite (nano HAP) and 1,450 ppm F; F group – toothpaste containing 1,450 ppm; and P group (placebo) – distilled water. Upon completion of the treatment period, the enamel microhardness and roughness profile were re-measured. **Results:** The demineralization procedure resulted in statistically significant reduction of SMH level in all the groups, and the mean post demineralization values were in the range of 49.7 VHN to 51.2 VHN. Remineralization therapies led to statistically significant increase of enamel SMH value ($P < 0.0001$). None of the groups reached their original baseline level of SMH following the remineralization therapy. (*Am J Dent* 2014;27:287-290).

CLINICAL SIGNIFICANCE: Both toothpastes, regular F and one containing nano-HAP, showed comparable effectiveness on enamel surface remineralization. Further investigation exploring total profile of carious lesions, beyond the superficial layer, is recommended.

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Introduction

The last two decades have seen continuous evolution on the epidemiology of dental caries. The changing demographics of caries support continued research in both general and at risk caries populations, even though rates of caries progression are generally slower than has been reported in the past. This manifests itself as shifts in the age in which caries process results in decayed surfaces requiring restoration. Thus, DMFS in 34 year-olds today is similar to the DMFS recorded in 17 year-olds some 20 years ago.[1-3] The existing presence of caries in the general population supports the need for further improvements in caries prevention therapies. Historically, fluoride treatment in various forms has been the primary approach for non-invasive caries treatment and prevention. In more recent years, however, various additional treatment options have been investigated. Among these is the application of calcium phosphate technologies.

The rationale behind the application of calcium phosphate technologies is because saliva support of fluoride in caries prevention is sometimes limited. The quantity of remineralization provided by the saliva along with buffering its capacity to reduce aciduric and acidogenic caries challenge is also limited. Research generally recognizes that calcium phosphate supplementation in various forms may uniquely contribute to reverse and arrest the caries process. Calcium phosphate therapies may be particularly useful when considered as options to locally repair or arrest developing lesions, thereby substituting for invasive restorative procedures.[4-6] The approach of lesion repair instead of restoration is further supported by improvements in detection techniques available to clinicians including Canary System, DIAGNOdent, QLF or Vista Cam.[7] Approaches utilizing calcium phosphate enhanced therapy have included casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) products (sold under the name Recaldent), calcium phosphate silicates (Novamin) as well as functionalized tricalcium phosphate (fTCP) agents.[8-11] Recently the nanohydroxyapatite (nano-HAP) has been proposed as a new remineralization alternative.[12-15] The application of particles of calcium phosphate instead of soluble calcium phosphate offers a unique approach, and presumably may have advantages in location and extent of remineralization protection. One might expect that particle applications would be most effective in treatment of surface lesions. This is due to obvious limitations in diffusivity and penetrability of treatments containing particle forms including nanoparticles. However, only limited literature supports the unique benefits of this potential approach.

This in vitro study compared the effect of a nano-HAP supplemented toothpaste to standard fluoridated toothpaste on surface remineralization of early carious lesions.

Material and Methods

Enamel sample preparation – Baseline measurements - The study was approved by the Ethics Committee of the Medical University of Warsaw (AKBE/9/12). Human extracted teeth, containing no apparent cracks, restorations or caries, and stored in 1% thymol solution, were used in the study. Ninety human enamel specimens (cylindrical sections), 6 mm in diameter were prepared under water cooled saw, and mounted in methacrylate polymer (Villacryl Hard Rapid[a]). The natural enamel surfaces were lightly smoothed by final surface polishing with 3.0 μm gamma alumina. Following sample preparation, base-

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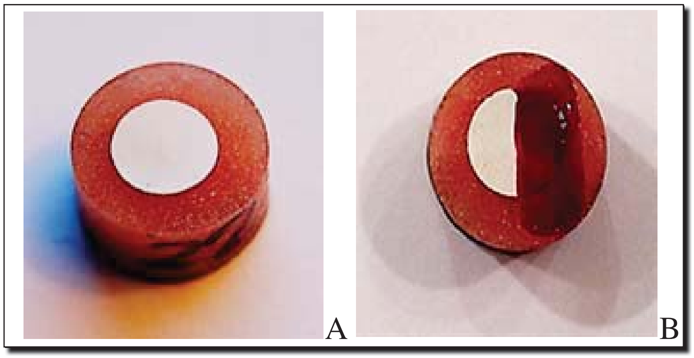

Fig. 1. **A.** Enamel samples mounted in discs of methacrylate polymer. **B.** Enamel samples prepared for pH cycling protocol, with approximately half the surface covered with a protective coating of nail varnish.

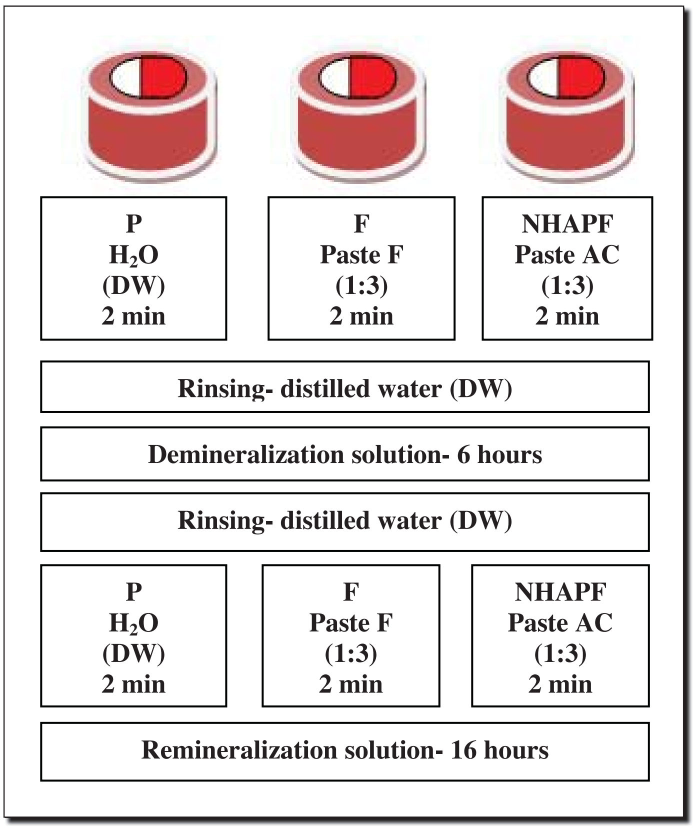

Fig. 2. Diagram of the pH-cycling protocol.

line measurements of surface hardness and surface roughness were performed. Hardness measurements were carried out using a Shimadzu HMV-2000[b] hardness tester with a Vickers diamond, under a load of 200g for 10 seconds. For each specimen, five random points were tested and the values were averaged. Enamel surface topography and roughness were qualitatively and quantitatively (mean Ra value) examined using a profilometer (PGM-1C[c]). Average surface roughness values were calculated for each specimen, and those exhibiting mean enamel surface microhardness (SMH) between 280-320 VHN were accepted for the study.

Demineralization pre-cycling measurements and treatment stratification - Following baseline measurements, subsurface enamel lesions were created through 96 hours of demineralization, according to the Carbopol method as described by White.[16] Following demineralization, specimens were reassessed by both surface hardness analysis and surface profilometry. Ninety samples were stratified to three treatment groups based on their surface hardness values (N= 30):

(1) NHAPF - Apa Care[d] toothpaste (1% nano-HAP + 1,450 ppm F/ NaF).

(2) F - Blend-a Med[e] toothpaste (1,450 ppm F/NaF).

(3) P- Placebo

Enamel lesion pH-cycling treatment protocol - After demineralization, one half of each specimen was covered with a protective nail varnish (Inglot Nail Enamel, Poland) (Fig. 1). The exposed surface was subjected to a 3-week cycle of pH cycling demineralization-remineralization challenges, as described by Featherstone et al.[17] This procedure, including 6 hours demineralization, 16 hours remineralization, and 2×/day 2-minute treatment with dentifrice slurries (Blend-a Med toothpaste, 25wt.% slurry - dentifrice in water) (Fig. 2). Upon completion of the pH cycling procedure, the enamel microhardness and roughness profiles were re-evaluated and compared between treatments.

Statistics - The statistical analysis was performed using the Statistica[f] 9.0PL software. In the subsequent stages of analyses Kołmogorow-Smirnow test, Shapiro-Wilk test, Mann-Whitney test, Kruskal-Wallis ANOVA test and Wilcoxon test were used.

Table 1. Mean SMH values at different timepoints.

Treatment	Baseline (B) VHN (SD)	Caries VHN (SD)*	pH cycling (R) VHN (SD)	b	Δ VHN (R-C) (SD)
NHAPF	304 (10.7)	51.2 (7.8)	188.7 (16.2)	B vs. C P< 0.0001 C vs. R P< 0.0001 B vs. R P< 0.0001	137.5 (13.2)
F	304.2 (11.9)	50.4 (9.0)	166.1 (13.8)	B vs. C P< 0.0001 C vs. R P< 0.0001 B vs. R P< 0.0001	115.7 (11.4)
P	303.9 (11.44)	49.7 (8.9)	46.4 (7.0)	B vs. C P< 0.0001 C vs. R ns	-3.3 (5.9)
a	ns	ns	P< 0.0001		

a Comparisons between treatment groups in the subsequent stages of research - ANOVA Kruskal-Wallis test.

b Comparisons of SMH values within groups - Wilcoxon test.

* Hardness values after the caries challenge were all significantly lower than baseline hardness. Specimens randomized to each treatment group were not significantly different for post caries hardness.

Results

The results of the Vickers measurements are shown in Table 1. Prior to demineralization, SMH of specimens were not statistically different between treatment groups. After the demineralization procedure, a statistically significant reduction of SMH level was observed in all of the groups, and the mean values ranged from 49.7 VHN to 51.2 VHN (Wilcoxon test, P< 0.0001). Comparing the mean values of SMH between all treatment groups after caries challenge, no significant differences were observed (ANOVA Kruskal-Wallis test, not significant). Application of the pH cycling treatment regimen resulted in a non-significant three unit decrease in the SMH of the placebo group (Wilcoxon test, not significant). The pH cycling treatment regimen produced an increase in mean enamel SMH value in both the NHAPF and F groups (Wilcoxon test, P< 0.0001). Though increased, the mean SMH values after pH cycling for the NHAPF and F treatment groups

Table 2. Mean Ra values obtained in each group at different phases of the experiment.

Treatment	Baseline (B) Mean Ra (SD)	Caries Mean Ra (SD)*	pH cycling (R) Mean Ra (SD)	b	Δ Ra (R-C) (SD)
NHAPF	0.08 (0.009)	0.09 (7.8)	0.07 (0.009)	B vs. C P< 0.001 C vs. R P< 0.0001 B vs. R P< 0.001	-0.02 (0.005)
F	0.08 (0.009)	0.10 (0.10)	0.09 (0.007)	B vs. C P< 0.0001 C vs. R P< 0.001 B vs. R P< 0.0001	-0.005 (0.003)
P	0.08 (0.01)	0.09 (0.02)	0.12 (0.02)	B vs. C P< 0.0001 C vs. R P< 0.0001 B vs. R P< 0.0001	0.02 (0.01)
A	P< 0.01	ns	P< 0.0001		

a Comparisons between treatment groups in the subsequent stages of research - ANOVA Kruskal-Wallis test.
b Comparisons of Ra values within groups - Wilcoxon test.
* Roughness values after the caries challenge were all significantly higher than baseline roughness. Specimens randomized to each treatment group were not significantly different for post caries roughness.

did not reach the baseline level (Wilcoxon test, P< 0.0001).

The results of surface roughness measures are shown in Table 2. Baseline measures showed specimens had a high degree of surface polish with Ra measures approximately 0.07 units. Following demineralization, specimens exhibited some degree of surface roughening, increasing to roughly 0.095 units (Wilcoxon test, P< 0.0001). Even though the treatments were stratified based on surface hardness, post demineralization surface roughness values for each of the allocated treatments were also found not to differ between groups (ANOVA Kruskal-Wallis test, not significant).

Following pH cycling, treatment groups exhibited unique and different changes with respect to surface roughness. Significant increase of surface roughness from 0.0945 to 0.1165 units (Wilcoxon test, P< 0.0001) was observed in the placebo group. The positive control with regular fluoride toothpaste (Group F) showed a slight but significant decrease in roughness following the pH cycling, from 0.0965 to 0.0914 units (Wilcoxon test, P< 0.001).

The NHAPF group showed a significant reduction of enamel roughness after the treatment, decreasing from 0.0955 to 0.0741 units (Wilcoxon test, P< 0.0001). The NHAPF group was the only treatment which exhibited smoothing during treatment and pH cycling.

Discussion

This study examined the effect of application of a toothpaste including nano-HAP and fluoride on carious enamel surface properties during pH cycling. Treatment effects were compared to a placebo and standard fluoride dentifrice control. The present study focused on evaluation only on surfaces of lesions since these are the areas where topical calcium phosphate nanoparticles might be expected to exert an effect.

Overall, the study results suggested additive benefits of nano-HAP incorporation into fluoridated dentifrice on carious lesion surfaces. Compared to the standard fluoride control, the nano-HAP fluoride dentifrice produced directional increase in surface hardness and statistically significant decrease in surface roughness. The fluoride control dentifrice in this study produced significant increase in lesion hardness, though negligible decreases in lesion surface roughness. These results suggest not only differences in the type of repair provided by nano-HAP supplementation, but also an additive effect for the incorporation of nanohydroxyapatite to the surface of lesions.

Those additive effects to fluoride could provide supplemental repair or protection against further caries development. Calcium phosphate may be beneficial to areas or patient populations where salivary enhancement of fluoride effects are not sufficient. For remineralization, fluoride requires a source of calcium phosphate which is naturally present in saliva and in plaque fluid. However, in cases of continued caries lesion development, these levels may not be adequate. The effect of nano-HAP might be expected to be surface specific and indeed results here are suggestive of this. While increased, the SMH measures were only directionally elevated. The microhardness of lesions is affected by structures below the lesion surface extending typically 2-3 times the actual indent depth. On the other hand, the surface roughness of nano-HAP dentifrice treated lesions was significantly decreased. These combined observations support the ability of the nano-HAP to integrate into the enamel carious lesion surface structure which is of itself valuable. The results of the present study support the hypothesis of Lata et al,[5] who suggested that the efficiency of mineral formulas used as an adjunct to fluoride products and varnishes of high fluoride concentration is more pronounced on enamel surface. The results also specifically support observation of nano-HAP toothpaste by Möller & Schröder.[18]

It would be expected that deposition of apatites initiated by the remineralization process caused the development of the highly mineralized external layer. This zone might be hypothesized to reduce demineralization progress, inhibiting diffusion of acids into deeper areas of enamel. Naturally, this deposition could block diffusion of mineral ions, thus constraining the subsurface enamel zone recrystallization. The problem of blocking the mineral ion diffusion into deeper areas of enamel by the superficial enamel layer has been discussed in many papers.[19-23] Taking into account the dynamic rate of the carious process and the necessity of constant control of agents conducive to demineralization and remineralization, it is worthwhile to investigate if reconstruction, limited to the superficial enamel layer, is sufficient and satisfactory for caries prevention and treatment. Therefore, further studies in this area, with analysis of the entire carious lesion profile, including body of the lesion area, should be undertaken to evaluate the total remineralization potential of nano-HAP containing therapies. This kind of analysis will be especially helpful for the development of optimal treatment plans in groups with high caries risk, including patients wearing orthodontic braces or those suffering from decreased saliva secretion.

a. Zhermack, Badia Polesine, Rovigo, Italy.
b. Shimadzu, Kyoto, Japan.
c. IOS, Cracow, Poland.
d. Cumdente, Tübingen, Germany.
e. Procter & Gamble, Cincinnati, OH, USA.
f. Dell Software, Aliso Viejo, CA, USA.

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