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Efficacy of various nano-hydroxyapatite dentifrices on remineralization potential in comparison with fluoride toothpaste on artificially-induced caries in extracted teeth

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Introduction

At 96% inorganic material, dental enamel is the most mineralized structure in the human body⁶. 90-92% of this inorganic material in enamel consists of hydroxyapatite¹¹. Hydroxyapatite is a complex crystalline form of calcium phosphate expressed in formula as $\text{Ca}_{10}(\text{PO}_4)_6\text{2}(\text{OH})$. Hydroxyapatite is also found, albeit in lower quantities, in bone, dentin, and cementum⁶.

Numerous empirical and clinical findings have shown hydroxyapatite to be safe and effective in both medical and dental surgeries¹¹. Hydroxyapatite coatings applied to titanium implants are marketed in both orthopedics and dentistry^{7,11}. Additionally, hydroxyapatite has been favored as a regenerative material for grafting in periodontal procedures, endodontic apicoectomies, and maxillofacial surgeries¹¹.

With the advent of nano-sized particle technology, nano-hydroxyapatite (n-HA) has slowly begun to take center stage in dental research. Nano-hydroxyapatite is a general term for any form of hydroxyapatite with a particle size from 50-1000 nm¹¹. Bioactive and bioceramic ingredients, including n-HA, have recently dominated the “buzz” in the dental community⁴. n-HA has also been researched as an alternative to larger particle hydroxyapatite in grafting and coated implants as well as an anti-erosion ingredient for acidic drinks¹¹.

The evidence for hydroxyapatite’s use as an anticaries agent spans decades. The first major experimentation with hydroxyapatite began with the National Aeronautics and Space Administration (NASA) as a regenerative agent for bone and tooth mineral loss in zero gravity. In 1970, the Sangi Co. Ltd (Japan) acquired the rights from NASA to extend the applications to a commercial market. Sangi became the first company worldwide to release a n-HA toothpaste (Apadent) specifically marketed as anti-caries and for the remineralization of surface enamel in 1978. In 2006, the first microcluster hydroxyapatite toothpaste appeared on the market in Europe^{11,2}. In 1993 in Japan and 2015 in Canada, hydroxyapatite (including n-HA) was officially approved as an anti-caries and remineralization ingredient in toothpaste².

Multiple studies have found compelling evidence for the efficacy of n-HA toothpaste by evaluating the remineralization potential of hydroxyapatite toothpastes utilizing microhardness testing. In these studies, extracted teeth with artificially-induced demineralized lesions were subjected to microhardness testing before and after cserial application of various dentifrices, including hydroxyapatite toothpastes. In 2019, Manchery et al. compared n-HA toothpaste with fluoride and NovaMin (5% calcium

sodium phosphosilicate, similar to amorphous calcium phosphate) using microhardness to determine the efficacy of remineralization potential in each dentifrice group. Manchery concluded the n-HA “dentifrice produce significantly better results compared to fluoride- and NovaMin-containing dentifrice”⁸. Similarly, microhardness study evaluating remineralization potential published in 2021 by Juntavee et al. compared n-HA toothpaste with fluoride and tricalcium phosphate (TCP) toothpaste. Their results showed greater remineralization potential with n-HA toothpaste by comparison⁷.

The University of Texas Health San Antonio by Amaechi et al. published a study in the British Dental Journal in 2019 that showed intriguing results. Unlike the two previously mentioned studies, this one utilized an intra-oral appliance model where the participants had enamel blocks with artificial carious lesions fixed in their mouths via an appliance. Using this model, remineralization potential of 10% microcluster hydroxyapatite toothpaste was compared with 500ppm amine fluoride toothpaste. While this concentration is not equivalent to most fluoridated toothpastes on market in the US, Amaechi et al. found that hydroxyapatite “achieved comparable efficacy” and “confirmed to be equal to the fluoride toothpaste in this study” in terms of remineralization of tooth structure².

The rise of single-use plastics has become a global crisis. The British Dental Journal concluded that 300 million tubes of toothpaste are discarded each year¹. Most of these tubes cannot be recycled even if the customer desires to do so. In 2020, Colgate launched its first recyclable toothpaste tube—a process that took five years of research and development⁵. Tom’s of Maine launched its first recyclable tube on the market a year prior in 2019¹³. Considering that only 9% of all plastic ever produced has successfully been recycled and only 8% has been incinerated, it is apparent that additional innovations in toothpaste packaging are needed to promote environmental sustainability for oral hygiene products¹⁰.

One trend that has arisen to address the toothpaste packaging dilemma is compressed toothpaste tablets packaged in glass bottles or aluminum tins. A basic internet search will reveal a multitude of brands marketing these tablets. While several of these products contain fluoride on the international market, this author is unaware of any to date in the US. At least one company, however, has marketed tablets containing n-HA. This author is also unaware of any studies comparing the effectiveness of toothpaste tablets of any form to either hydroxyapatite or fluoride toothpastes

Objective

To compare the remineralization potential of nano-hydroxyapatite dentifrices with fluoride in both traditional toothpaste and toothpaste “tablet” dentifrices utilizing microhardness testing on artificially-induced carious lesions in extracted teeth.

Hypothesis

Regardless of storage composition as a tablet or toothpaste, nano-hydroxyapatite and fluoride dentifrices will exhibit similar remineralization potential when subjected to microhardness testing.

Methods and Materials

50 previously extracted human teeth were collected for use in the experimental sample. Teeth with obvious caries, dental restorations, evidence of demineralization, cracks, or any other signs of enamel compromise will be excluded. Teeth were stored in a 10% formalin solution until testing.

The sample teeth were decoronated and then sectioned with a flat surface established so that the buccal or lingual smooth surface was parallel to the table. This produced 100 working samples to be divided into groups for testing. Black nail varnish (Revlon) was placed over the exposed enamel. Then a flat, 3mm x 3mm window at the buccal/lingual height of contour was prepared with diamond discs to parallel the table. This flat surface of exposed enamel was the subject of Hardness testing in each sample.

Dulbecco's phosphate buffered saline (DPBS, ThermoFisher) was selected for the storage medium throughout testing. DPBS was determined to be an ideal solution due to its baseline pH of 7.0 with chemical composition of CaCl_2 , MgCl_2 , KCl , KH_2PO_4 , NaCl , and Na_2HPO_4 , which closely mirrors the electrolyte composition and pH of human saliva at rest. To induce the artificial carious lesions, the windows of exposed enamel were treated with 40% phosphoric acid etch for 45 seconds each.

After preparation, each tooth was numbered and randomly divided into 5 groups of 20 each. Group 1 (*FP*) was treated with an ADA approved, over-the-counter fluoride toothpaste (Colgate Complete 0.454% sodium fluoride). Group 2 (*HAP*) was treated with n-HA toothpaste (Apagard Premio, 10% n-HA). Group 3 (*FT*) was treated with fluoride toothpaste tablets (Bite Toothpaste Bits, Sodium Monofluorophosphate, 0.76%). Group 4 (*HAT*) was treated with n-HA toothpaste tablets (Bite Toothpaste Bits, undisclosed % n-HA). Group 5 (*nt*) was an untreated control group.

Throughout testing, all samples were stored in individual glass vials with 5mL of DPBS with the solution changed out before each new application of surface treatment. To mimic an oral hygiene regimen, all samples except the control group underwent surface treatment treated with the four dentifrices twice daily for 2 minutes. Excess dentifrice was wiped off the teeth with gauze but not rinsed and each tooth was placed back into its respective glass vial with a new 5mL solution of DPBS.

Surface microhardness was evaluated utilizing a Knoop Hardness Indenter (Leco LM300AT) with a 500g/15 second setting. Each specimen was tested prior to induction of the artificial caries (H_B), after caries induction (H_D), and after completion of the pH cycling (H_R).

Data Analysis

Statistical significance for all statistical tests was declared at $P < 0.05$. Data were analyzed using SPSS 25.0 (IBM, Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normality of the data distributions for continuous data. Accordingly, measures of central tendency are presented as means with associated standard deviations. A One-way Analyses of variance (ANOVA) was conducted to check for differences in between groups with respect to sample hardness before and after demineralization as well as the hardness after remineralization. Post-hoc assessment of

significant results were accomplished using Tukey's test. The Levene statistic was used to confirm the assumption of homogeneity of variances. Consequently, assessment of sample hardness recovery (HR) and remineralization potential (RP) was conducted using the Kurskal-Wallis test. Quantification of HR and RP was calculated based on formulas for surface hardness recovery (% HR) and remineralization potential (% RP) shown below as previously constructed by Juntavee et al.ⁱ

$$\% \text{ HR} = (H_R - H_D / H_B - H_D) \times 100$$

$$\% \text{ RP} = (H_R - H_R \text{ of } nt / H_D) \times 100$$

Results

Data were successfully collected from a total of 92 samples. Table 1 reports the mean hardness of samples by group. Although numerically, samples in the fluoride paste group showed the highest mean hardness (321.5MPa, SD = 46.0) before demineralization and samples from the fluoride tablet group had the lowest (308.9 MPa, SD = 20.5). ANOVA results indicated no statistically significant difference was present, $P = 0.55$. Similarly, analysis showed no statistical difference in mean hardness between groups after demineralization, $P = 0.29$. [Table 1 & Table 2]

A significant difference in mean hardness was found between the groups after remineralization, $P < 0.001$. Post-hoc analysis revealed that samples with no treatment had significantly lower hardness (208.0 MPa, SD = 15.3) compared to those treated with FP, HAP, FT, or HAT (all $P < 0.001$). However, no pairwise differences in hardness were noted between the treatment groups after remineralization (all $P > 0.05$). Similarly, examination of the % HR and % RP revealed no significant differences among treatment groups (both $P > 0.05$). [Table 3]

Discussion

Despite the evidence for hydroxyapatite in preventive dentistry, there appears to be considerable professional resistance towards any dentifrice ingredients for the purpose of anticaries other than fluoride and xylitol in the United States. The American Dental Association (ADA) only extends its seal of approval to toothpastes containing fluoride⁹. While the U.S. Food and Drug Administration (FDA) generally classifies hydroxyapatite to be considered safe as a substance, it offers no specifics to particle size or n-HA¹². To date, the FDA has not approved any toothpastes containing hydroxyapatite (nano- or micro-particle) for use as an anticaries agent.

Fluoride exposure remains the gold-standard dental material for anticaries and remineralization. There are numerous methods for fluoride exposure, including systemic (fluoridated water or dietary supplementation) and topical. The latter can be subdivided into professional application (varnish, gels, and pumice) and at-home application (toothpaste and mouth rinses)⁶.

In addition to inhibiting bacterial adhesion, fluoride exposure is effective due to its behavior when exposed to hydroxyapatite. The available fluoride ion undergoes a precipitation reaction with the outer enamel hydroxyapatite to form fluorapatite, which can remineralize non-cavitated lesions on the surface⁶.

Amorphous calcium phosphate (ACP) and tri-calcium phosphate products have also recently gained popularity on the market in a variety of forms. ACP and TCP toothpastes act as an adjunct to fluoride for enamel remineralization. Both ACP and TCP products are formulated to “convert to apatite and remineralize the enamel when it comes in contact with saliva”⁶. Hypothetically, this results in both direct remineralization as well as a catalyst to enhance the replacement mineralization precipitation of fluorapatite⁷. Unlike hydroxyapatite dentifrices, many of these products have gained ADA and FDA approval due to their use as an adjunct rather than an alternative to fluoride.

There has been a growing distrust of fluoride among the general population both in the United States and worldwide despite any significant evidence of harm at the levels used for anticaries³. As such, many fluoride-free toothpastes and other dentifrices have grown in popularity on the market. The safety and effectiveness of fluoride are not disputed by this author. However, it is worth recognizing that if alternative dentifrice ingredients can produce results comparable to fluoridated toothpaste, then those products should not be dismissed or disregarded solely on their absence of fluoride. Additionally, young children and pregnant women represent groups to which decreased fluoride exposure is recommended. Fluoride alternative dentifrices such as those containing n-HA may represent an effective tool for clinicians, particularly for those treating high caries risk patients in these demographics.

There was no evidence of negative influence by suspending hydroxyapatite or fluoride in a desiccated, compressed tablet when compared with traditional toothpastes. Therefore, while toothpaste tablets may not offer a distinct clinical advantage, there appears no evidence of harm in their use. Furthermore, toothpaste tablets may offer logistical and military advantage. Toothpaste tablets are lightweight, shelf stable, and possess a small storage footprint. They could be used easily for oral hygiene in large scale combat operations and contingency operations. They are TSA compliant and can be rationed out for each toothbrushing interval (one tablet per oral hygiene interval). This author can imagine toothpaste tablets being packaged and included in military food rations, such as the meal-ready-to-eat (MRE).

Conclusion

While fluoride has a proven track record, the growing evidence for hydroxyapatite as an alternative is difficult to ignore. These findings suggest that the efficacy of toothpaste tablets can be comparable to conventional dentifrices in their remineralization potential. In addition to remineralization potential, fluoride’s role of fluorapatite deposition is valued by dental professionals for its supposed superior resistance to future demineralization compared to hydroxyapatite. Future studies could be focused to repeat this study with an additional round of demineralization and subsequent microhardness testing. If the fluorapatite groups proved more resistant to a second round of demineralization, then fluoride dentifrices would still be favored by this

author over hydroxyapatite dentifrices. More research is also needed to establish the efficacy of alternative dentifrices in other aspects such as antibacterial properties and abrasivity.

The findings of this study alone cannot definitively prove the safety of hydroxyapatite. However, this study adds data to support a growing body of evidence that hydroxyapatite is at least equally effective at remineralizing caries in non-cavitated enamel lesions.

Likewise, these findings suggest that toothpaste in the form of a compressed tablet does not negatively influence its ability to influence remineralization potential so long as the tablet contains a remineralizing agent.

There are clear benefits to hydroxyapatite as an ingredient in toothpaste as well as toothpaste tablets. Clinicians would be well advised to be aware of these products as they gain popularity. While this author is not ready to advocate for the use of hydroxyapatite over fluoride exclusively, a toothpaste that offers fluoride and hydroxyapatite in combination is an enticing concept. Similarly, the already fluoride averse patient and “zero waste” conscious patient, these seemingly novel dentifrice applications appear to be viable parts of safe and effective oral hygiene.

Military Significance

Hydroxyapatite appears to have a promising use as both an adjunct to fluoride in high caries-risk military service members as well as an alternative for pregnant service members and children of service members. Additionally, if hydroxyapatite toothpaste tablets prove efficacious in remineralization potential, their shelf-stable nature would prove particularly well-adapted to extended periods in austere environments such as combat zones and stability operations.

Institutional Relevance

Postgraduate Dental College Gap Analysis, II. Prevention and Safety, A. Disease Prevention:

Employment of anticariogenic materials and devices for preventive purposes

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References

1. 300 million toothpaste tubes go to landfill. (2021). *British Dental Journal*, 230(7), 390-390. <https://doi.org/10.1038/s41415-021-2926-y>
2. Amaechi, B. T., Abdulazees, P. A., Alshareif, D. O., Shehata, M. A., Lima, P. S., Abdollahi, A., Kalkhorani, P. S., & Evans, V.. (2019). Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. *BDJ Open*, 5(1). <https://doi.org/10.1038/s41405-019-0026-8>
3. Chi DL. Parent Refusal of Topical Fluoride for Their Children: Clinical Strategies and Future Research Priorities to Improve Evidence-Based Pediatric Dental Practice. *Dent Clin North Am*. 2017;61(3):607-617. doi:10.1016/j.cden.2017.03.002
4. Goldstep, F. (2018). *Bioactivity In Restorative Dentistry: A User's Guide*. Retrieved 02 September 2021 from <https://www.oralhealthgroup.com/features/bioactivity-restorative-dentistry-users-guide/>
5. Guzman, F. Ries, B. (2020). *Colgate finally launched its recyclable toothpaste tube. It's made from the same type of plastic as milk jugs*. CNN. Retrieved 27 August 2021 from <https://www.cnn.com/2020/01/15/business/colgate-recycle-toothpaste-tube-trnd/index.html>
6. Heymann, H., Swift, EJ., Ritter, AV., Sturdevant, CM. *Sturdevant's Art and Science of Operative Dentistry*. 6th ed. St. Louis Mo: Elsevier/Mosby; 2013.
7. Juntavee, A., Juntavee, N., & Hirunmoon, P.. (2021). Remineralization Potential of Nanohydroxyapatite Toothpaste Compared with Tricalcium Phosphate and Fluoride Toothpaste on Artificial Carious Lesions. *International Journal of Dentistry*, 2021, 1–14. <https://doi.org/10.1155/2021/5588832>
8. Manchery, N., John, J., Nagappan, N., Subbiah, G. K., & Premnath, P. (2019). Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative *in vitro* study. *Dental research journal*, 16(5), 310–317.
9. *Oral Health Topics, Toothpaste*. (2021). American Dental Association. Retrieved 27 August 2021 from <https://www.ada.org/en/member-center/oral-health-topics/toothpastes>
10. Parker, L. (2018). *Here's how much plastic is littering the Earth*. National Geographic. Retrieved 26 August 2021 from <https://www.nationalgeographic.com/science/article/plastic-produced-recycling-waste-ocean-trash-debris-environment>
11. Pepla, E., Besharat, L. K., Palaia, G., Tenore, G., & Migliau, G. (2014). Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: a review of literature. *Annali di stomatologia*, 5(3), 108–114

12. Rupp, R. *Nanoparticles: Should We Be Worried?* National Geographic. Retrieved 22 August 2021 from <https://www.nationalgeographic.com/culture/article/nanoparticles-and-you?loggedin=true&rnd=1685668334447>

13. *Tom's of Maine Brings First-of-its-Kind Recyclable Toothpaste Tube to the Oral Care Aisle.* (2019). Retrieved 27 August 2021 from <https://www.tomsofmaine.com/news/natural-toothpaste-now-with-recyclable-tube>

Abbreviations & Acronyms

HA= hydroxyapatite
n-HA= nano-hydroxyapatite
ACP= amorphous calcium phosphate
ADA= American dental association
FDA=food & drug administration
TCP= tri-calcium phosphate
DPBS= Dulbecco's phosphate buffered solution
KMN= surface microhardness
VLC- visible light cure
H_B= hardness before artificial caries induction
H_D= hardness after artificial caries induction
H_R= hardness after remineralization
RP= remineralization potential
FP= Group 1, fluoride toothpaste
HAP= Group 2, n-HA toothpaste
HAT= Group 3, nHA tablets
nt= Group 4, no treatment

Table 1.

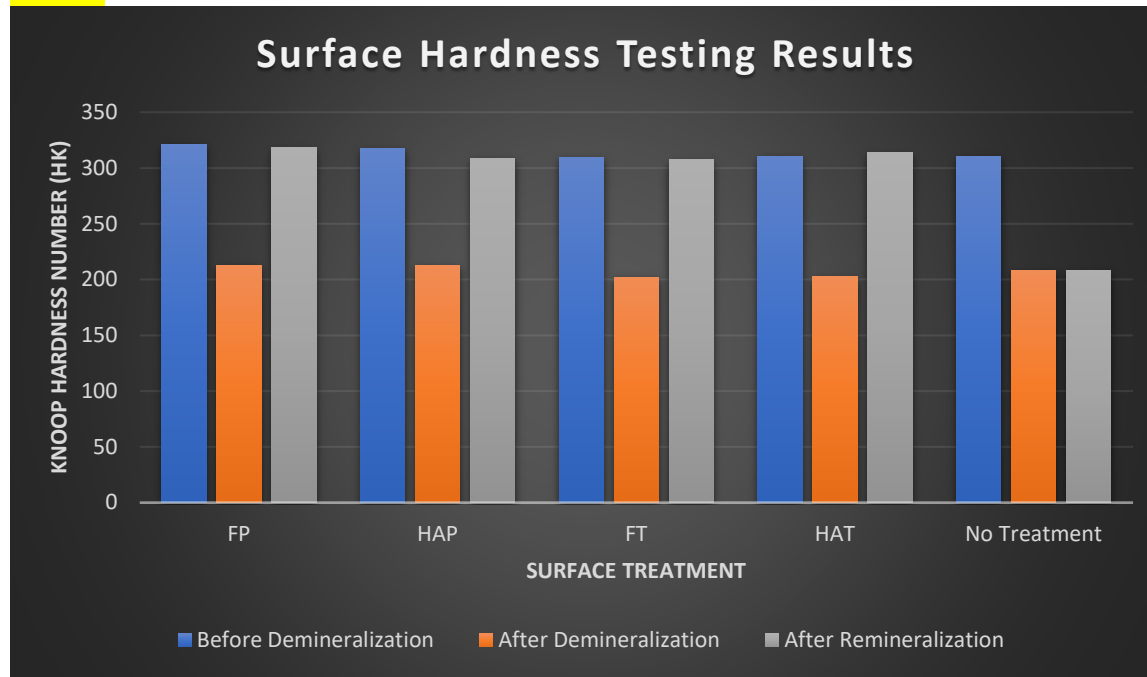


Table 2.

Condition	FP (fluoride paste), n=15	HAP (hydroxyapatite paste), n=17	FT (fluoride tablets), n=20	HAT (hydroxapatite tablets), n=20	No treatment, n=20
Before Demineralization	321.5 (46.0)	317.4 (24.2)	309.4 (24.3)	308.9 (20.5)	310.3 (11.5)
After Demineralization	212.9 (24.0)	212.8 (16.7)	202.0 (19.1)	202.5 (21.8)	208.1 (18.5)
After Remineralization	318.0 (30.8)	308.8 (14.3)	307.4 (26.1)	313.5 (20.0)	208.0 (15.3)
Hardness Recovery	107.5 (58.8)	92.8 (14.3)	102.2 (32.2)	104.9 (12.0)	-0.8 (5.3)
Remineralization Potential	52.8 (18.8)	47.2 (4.5)	49.3 (12.7)	52.4 (10.4)	-0.5 (6.5)
