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Surface enamel remineralization by biomimetic nano hydroxyapatite crystals and fluoride ions effects

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The use of specific remineralizing agents in toothpastes may help to prevent caries and treat human enamel. In this study, applied nanotechnologies were used to develop toothpastes with remineralizing properties. Hydroxyapatite nanocrystals, with size, morphology, chemical composition comparable with that of enamel, were synthesized by wet chemical method. The remineralizing effect was studied with a scanning electron microscopy (SEM) of enamel previously demineralized. The SEM images demonstrated differences in micro surface at each step. The rate of remineralization seems to be compatible with the development of toothpastes with remineralizing effect. In conclusion, toothpaste containing nano-sized hydroxyapatite has the potential to remineralize an incipient caries lesion. In addition, the use of fluoride in toothpaste contains nano- hydroxyapatite might promote formation of nano fluoro hydroxyapatite in enamel surface.

Key words: Hydroxyapatite, Nanocrystals, Human enamel, Remineralization, Toothpaste, Dental materials.

Introduction

It is now well established that dental caries in its early stage of formation (non-cavitated) can be remineralized [1, 2], and this remineralization can be facilitated by such agents as fluoride, delivered via either mouth rinse or dentifrice [3, 4]. However, extensive use of fluoride mainly in the form of a dentifrice has contributed to a rising incidence of dental fluorosis, particularly in preschool children, due to chronic ingestion of these products [5]. Hence, there is a need for an alternative caries remineralizing and preventive agent as effective as fluoride, but without its harmful effects.

Recent research has emphasized the relevance and necessity of biomimetic oral health products containing nano-sized hydroxyapatite particles in modern preventive dentistry [6-9].

Nano-hydroxyapatite (n-HA) is considered one of the most biocompatible and bioactive materials, and has gained wide acceptance in medicine and dentistry in recent years [10]. Nano-sized particles have similarity to the apatite crystals of tooth enamel in morphology and crystal structure [11]. Recently, a few reports have shown that n-HA has some potential to repair dental enamel. Nanoparticle HA–containing toothpastes were first introduced and tested in Japan in the 1980s (e.g. Apadent, Apagard, and others by Sangi Co., Ltd., Tokyo). Since then there have been several studies, including field trials, to test their efficacy in caries prevention, leading to their approval as anti-caries agents by the Japanese Government in 1993. These studies, however, were carried out at the manufacturer's request, and the results were published only in Japanese-language journals [12, 13].

The aim of this in vitro study was to evaluate the effect of Nano-Hydroxyapatite toothpaste on the remineralization of human enamel.

Experimental Details

The n-HA hydrogel product used in this study for the damaged enamel surface remineralization has been obtained through the sintering reaction, at 40 degrees and uniform dispersed in an aquous carboxy methyl cellulose gel. We used the reaction between H_3PO_4 and $Ca(OH)_2$ both in aqueous phase, at constant pH of 7.5 units, through a continuous process perfected in a microreactor assembly equipped with static mixers. The reagents were analytical pure: phosphoric acid from MERCK (Germany); calcium hydroxide from MERCK (Germany); carboxymetilcellulose for alimentary use Sigma-Aldrich (USA), and de-ionized water.

Preparation of demineralizing solution

The buffered de-mineralizing solution was prepared using analytical grade chemicals and de-ionized water. The demineralizing solution contained 2.2 mM calcium chloride, 2.2 mM sodium phosphate, and 0.05 M acetic acid; the pH was adjusted with 1 M potassium hydroxide to 4.4 (all reagents were purchased from

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MERK (Germany)).

Erupted permanent teeth were cleaned by storing them in distilled water for at least 24 hours after their root and pulp had been removed. The proximal side was sectioned with a diamond wheel disk and enamel pieces were embedded in an epoxy resin for the fixation of the tooth surface. The enamel samples were then smoothed and polished to mirror flatness on a lapidary wheel using wet abrasive sandpaper up to 1000 grit for SEM evaluation.

Preparation of artificial carious lesions

The enamel samples were immersed in the demineralization solution at room temperature with constant stirring (200 rpm) for 12 hours. Subsequently, the enamel samples were carefully washed using tap water to remove any excess acid.

Toothpaste preparation

The toothpaste slurries for remineralization were prepared by mixing 100 grams of toothpaste in 100 ml distilled water. One group (Group A) contained nano-sized hydroxyapatite and fluoride (Sodium monofluorophosphate 1450 ppm), and the other (Group B) contains nano-sized hydroxyapatite excluded fluoride. Because fluoride strongly affects the remineralization of demineralized teeth, toothpastes containing nano-sized hydroxyapatite excluding fluoride were used as the control [14]. Remineralization was carried out at room temperature in such way that the remineralizing solution (200 ml) was continuously stirred (200 rpm) and the enamel sample remained static. The deposition of nano-sized hydroxyapatite on the demineralized enamel surface was induced by immersing the specimen in the toothpaste slurries for 24 (remineralization I) or 48 (remineralization II) hours. After remineralization, the enamel samples were carefully washed in tap water and dried prior to analysis.

In order to evaluate the remineralization effect, Scanning Electron Microscope (SEM) was also used to examine the micro surface of the specimens of each step.

Results and Discussion

XRD analysis

The structural analysis of sample was done by the powder X-ray diffraction. The XRD patterns of the synthesized nano hydroxyapatite are shown in Fig. 1. The XRD pattern of nano hydroxyapatite shows sharper peaks which indicate better crystallinity. The peak positions are in good agreement with the JCPDS (896438). As can be seen, hydroxyapatite XRD patterns, with the diffraction peaks, obtained with d-spacing values of 2.82 Å, 2.79 Å and 2.72 Å. The results of XRD analysis obtained in the present investigation are in good agreement with the reported results [15].

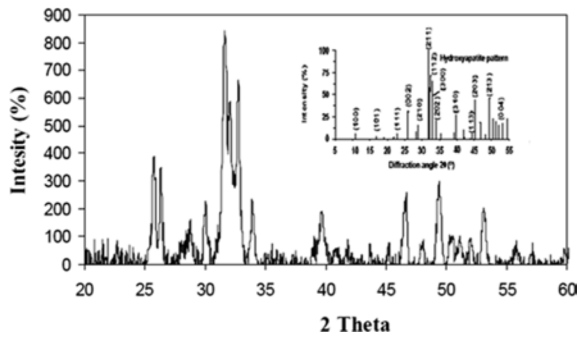

Fig. 1. XRD spectra of hydroxyapatite nanoparticles.

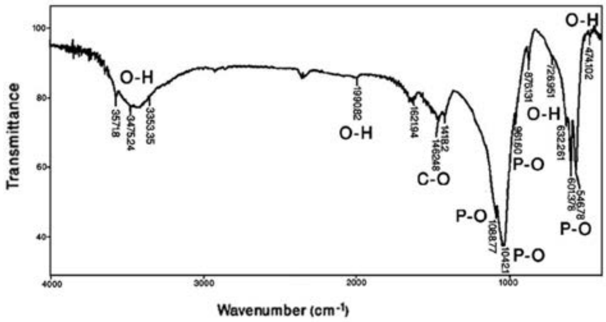

Fig. 2. FTIR spectrum of nano- hydroxyapatite.

FTIR analysis

Functional groups associated with hydroxyapatite were identified by FTIR spectroscopy. The FTIR spectrum of the prepared sample is given in Fig. 2. The ion stretching vibration around 3568 cm^{-1} confirms the presence of a hydroxyl group. Likewise, the other stretching vibrations for carbonyl and phosphate groups were also observed as reported earlier [16]. The observed functional groups and their corresponding assignments are presented in Table 1.

The functional groups of the hydroxyapatite powder predicted from FTIR spectrum analysis is compared with the results of Choi et al. for the confirmation [17].

SEM analysis

The scanning electron microscope (SEM) was used for the morphological study of nanoparticles of hydroxyapatite. Fig. 3 shows the SEM image of the as prepared hydroxyapatite nanoparticles. The spherical

Table 1. Some important functional group assignments of hydroxyapatite nanoparticles.

Wave number (cm^{-1})	Stretching mode	Functional group
3571	Ion Stretching	OH^-
1462	Asymmetric stretching	CO_3^{2-}
1042	Asymmetric stretching	PO_4^{3-}
878	Out of plane bending mode	CO_3^{2-}
546	Asymmetric bending vibration	PO_4^{3-}

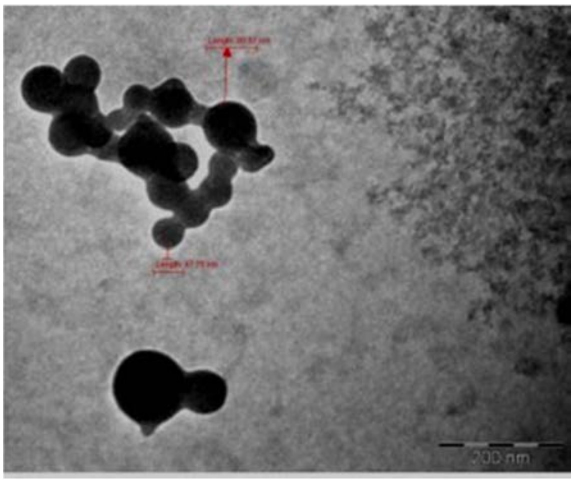

Fig. 3. TEM images of hydroxyapatite nanoparticles.

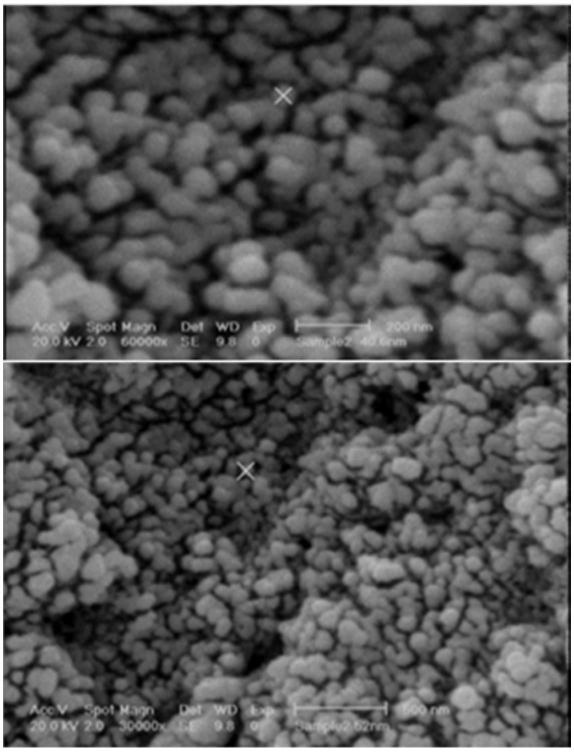

Fig. 4. Scanning electron microscopy (SEM) images of the hydroxyapatite nanoparticle.

shaped particles with clumped distributions are visible from the SEM analysis. The SEM images show the spherical shaped particles as confirmed by Ferraz et al. for reported results of hydroxyapatite nanoparticles [18].

TEM analysis

The structure and morphology of the samples were further confirmed by the TEM. TEM image of the prepared nano-hydroxyapatite, as shown in Fig. 4. The transmission electron microscopic analysis confirms the presence of the spherical shape morphology of the prepared hydroxyapatite nanoparticle with the particle

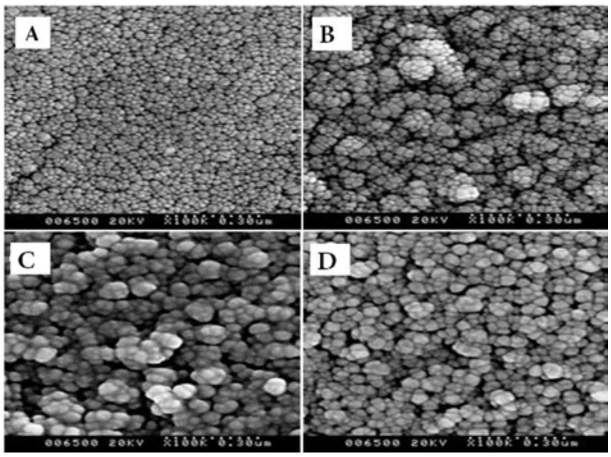

Fig. 5. Scanning electron microscopy (SEM) observations of (A) before deminerizing of dental enamel, (B) demineralized dental enamel surface, (C) demineralized dental enamel surface treated with toothpaste containing n-HA, (D) demineralized dental enamel surface treated with toothpaste containing n-HA + fluoride.

size of around 60 to 70 nm (Fig. 4). The particle size is also found to be in agreement with the report results [18].

Fig. 5 shows SEM images highlighting the change in the microstructure of the tooth surface at each step. Mineral loss was observed after demineralization and an intact enamel surface was observed after treating with the toothpaste slurry containing the n-HA for remineralization.

While the healing effects of fluoride-containing toothpastes are well established [19, 20], only limited literature is available for HA-containing toothpastes. As most of the research has been carried out in Japan, the findings have only been published in Japanese-language journals.

Toothpastes containing abrasive grade HA (micron-sized particles) are available in Japan and South Korea; however, investigators have only studied the abrasive or whitening characteristics of the toothpaste [21-23], rather than their remineralization efficacy.

The results of this study indicate that the n-HA toothpaste produced similar results to the positive control involving a fluoride toothpaste. Both of these toothpastes showed a similar ability to reduce the progress of demineralization, while simultaneously enhancing the remineralization of the artificial caries-like lesions. The supernatant obtained from the n-HA toothpaste slurry had the same pH as that of controls. It was free of fluoride and contained high calcium and phosphate ion concentrations, making the solutions highly supersaturated with respect to HA in enamel. In contrast to fluoride, nanoparticle HA is believed to affect the remineralization of tooth enamel at both the nanocrystalline and ionic levels. In our present study, supernatant of a toothpaste solution was used.

Groups treated with hydroxyapatite toothpaste revealed remineralized subsurface lesions compared to baseline, but without any hypermineralization. The used nano-sized particles (20 nm in size, with granular dimensions up to 100-150 nm) as well as the calcium arising from storage solution should have followed a concentration gradient (with the solution higher than the subsurface lesion), thus leading to a remineralizing effect in deeper lesion parts [24].

Conclusions

The prevention of tooth decay and the treatment of lesions are ongoing challenges in dentistry, and nanotechnology has been claimed as one of the most revolutionary approaches in this field. Interestingly enough, within the limitations of the present in vitro set up, the different nano-hydroxyapatite toothpastes revealed similar remineralizing capacities with enamel and dentine lesions. For enamel, higher remineralization effects could be achieved with n-HA toothpastes compared to the fluoride dentifrice. From the present outcome, we therefore speculate that nanohydroxyapatite in dental products might help to promote remineralization.

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References

1. J.M. ten Cate, J.D. Featherstone, Crit Rev Oral Biol. Med. 2 (1991) 283-296.
2. J.D.B. Featherstone, Aust Dent J. 53 (2008) 286-291.
3. F. Schafer, Caries Res. 23 (1989) 81-86.
4. J.S. Wefel, M.E. Jensen, P.T. Triolo, R.V. Faller, M.M. Hogan, W.D. Bowman, Am. J. Dent. 8 (1995) 217-220.
5. D.G. Pendrys, J.W. Stamm, J. Dent. Res. 69 (Spec Iss) (1990) 529-538.
6. D. Jumanca, A. Galuscan, A. Podariu, L. Ardelean, L. Cristina Rusu, REV. CHIM. (Bucharest) 64 (5) (2013) 555-556.
7. K. Onuma, K. Yamagishi, A. Oyane, J. Cryst. Growth 282 (2005) 199-207.
8. P. Tschoppe, D.L. Zandim, P. Martus, A.M. Kielbassa, J. Dent. 39 (2011) 430-437.
9. L. Li, H. Pan, J. Tao, X. Xu, C. Mao, X. Gu, R. Tang, J. Mater. Chem. 18 (2008) 4079-4084.
10. M. Hannig, C. Hannig. Nature Nanotechnology 5 (2010) 565-569.
11. J. Vandiver, D. Dean, N. Patel, W. Bonfield, C. Ortiz, Biomaterials 26 (2005) 271-283.
12. T. Kani, M. Kani, A. Isozaki, et al, JPN. Journal of the Japanese Society for Dental Health 38 (1988) 364-365.
13. T. Kani, M. Kani, A. Isozaki, H. Shintani, T. Ohashi, T. Tokumoto, JPN. Journal of the Japanese Society for Dental Health 39 (1989) 104-109.
14. M.Y. Kim, H.K. Kwon, C.H. Choi, B.I. Kim, Key Enggineering Materials (2007) 330-332.
15. E. Bouyer, F. Gitzhofer, M.I. Boulos, J. Mater. Sci. Mater. Med. 11 (2000) 523-531.
16. B. Cengiz, Y. Gokce, N. Yildiz, Z. Aktas, A. Calimli, Colloids and Surfaces A: Physicochemical and Engineering Aspects. 322 (1-3) (2008) 29-33.
17. D. Choi, K. Marra, P.N. Kumta, Materials Research Bulletin. 39 (2004) 417-432.
18. M.P. Ferraz, F.J. Monteiro, C.M. Manuel, J. Appl. Biomater. Biomech. 2 (2004) 74-80.
19. R.L. Karlinsey, A.C. Mackey, G.K. Stookey, Am J Dent. 22 (2009) 185-188.
20. J.M. ten Cate, H.E. Rempt, Caries Res. 20 (1986) 193-201.
21. A. Nakano, K. Fujita, S. Shinohara, JPN. Hiroshima Daigaku Shigaku Zasshi 15 (1983) 320-324.
22. T. Kodaka, M. Kobori, A. Hirayama, M. Abe, J. Electron Microsc. (Tokyo) 48 (1999) 167-172.
23. M. Niwa, T. Sato, W. Li, H. Aoki, T. Daisaku, J. Mater. Sci. Mater. Med. 12 (2001) 277-281.
24. T. Aoba, Oral Diseases 10 (2004) 249-257.