SEVEN publicações acadêmicas

Study of the potential of nano hydroxyapatite in dental remineralization: An ex vivo study

Crossref doi
https://doi.org/10.56238/interdiinovationscrese-001

Daiane Vitória Duarte Quandt
Dentistry Course, University of Joinville Region, Santa Catarina, Brazil.

Luiza Helena Rocha do Nascimento
Dentistry Course, University of Joinville Region, Santa Catarina, Brazil.

Daniela Cunha Coelho
Dentistry Course, University of Joinville Region, Santa Catarina, Brazil.

Michele Cristina Formolo Garcia
Chemical Engineering Course, University of Joinville Region, Santa Catarina, Brazil.

Daiara Floriana da Silva
DNPRIME do Brasil, Inovaparque - R. Paulo Malschitzki, 10 - Zona Industrial Norte, Joinville, Santa Catarina, Brazil.

Nelson Levandowski Junior
DNPRIME do Brasil, Inovaparque - R. Paulo Malschitzki, 10 - Zona Industrial Norte, Joinville, Santa Catarina, Brazil.

Kesly Mary Ribeiro Andrades
Dentistry Course, University of Joinville Region, Santa Catarina, Brazil.
E-mail: keslyribeiro@gmail.com

ABSTRACT
Introduction: Nanohydroxyapatite (nano-HAp) has been studied as a biomimetic material that promotes remineralization of the tooth surface and obliteration of dentinal tubules, being considered an alternative to the use of fluoride. In addition to the benefits to tooth structure and unlike fluoride, the accidental ingestion of nano-HAp as a component of toothpaste is not associated with any relevant systemic health risk. Objective: The aim of this study was to evaluate, in vitro, the potential of nano-HAp to remineralize the tooth structure, comparing three different formulations of toothpastes: pure synthetic nanohydroxyapatite, synthetic nanohydroxyapatite with fluoride and only fluoride, through electron microscopy scanning (SEM) and chemical analysis (EDS). Material and methods: A cross-sectional experimental study with a qualitative approach was carried out. Samples of teeth, third molars, cut into blocks, exposing enamel and dentin surfaces, were submitted to demineralization by immersion in 37% phosphoric acid for 30 seconds, followed by washing in running water. Subsequently, brushings were performed with the three toothpastes and in a control group, for 10 consecutive days. At the end of the experiment, a qualitative analysis was performed with Scanning Electron Microscopy (SEM) and chemical analysis (EDS). Results: It can be observed that in the control group there was no obliteration of the dentinal tubules and no change in the morphology of the dentin surface. In toothpastes with 5% nanohydroxyapatite and fluoride nanohydroxyapatite, mineral deposition on the dentin surface and obliteration of dentinal tubules can be observed, however, there is a more uniform deposition in toothpaste with 5% nanohydroxyapatite. In the fluoride toothpaste, it was possible to observe a mineral deposition, however, this was more subtle and only within some dentinal tubules, without altering the morphology of the dentin. Conclusion: It can be concluded that nanohydroxyapatite promoted a mineral deposition on the dentin surface and inside the dentinal tubules, demonstrating promising results in the remineralization of the tooth structure.

Keywords: nanohydroxyapatite, remineralization, toothpaste.

1 INTRODUCTION

Dental caries is a complex disease that affects the majority of the population regardless of gender, age and socioeconomic status. It is one of the most prevalent diseases worldwide and affects even developed countries with a well-established health system, such as Germany, USA and Australia [Paszynska et al. 2021] [27]. The demineralization of dental tissues is the main mechanism involved in the development of caries. As such, it is the result of complex interactions that occur between hard tissues, the microbial biofilm that colonizes surfaces, and nutrient intake [Esposti et al. 2021] [11].

Fluoride is considered the gold standard in Brazil as an agent that inhibits demineralization processes. Its effects are well known and targeted at reducing the metabolic and physiological pathways of acidogenic microorganisms. The use of fluorides for the prevention and control of carious processes is shown to be an effective method, with the use of topical fluoride, present in the formulation of toothpastes or under various forms of professional application, a preventive measure of paramount importance. "The recommended level of fluoride for daily use is 0.05 - 0.07mg F/Kg/day, which is considered to be of great help for the prevention of dental caries, acting on remineralization" [Alvarez, 2009] [1]. However, the administration of fluoride-containing treatments must be carefully balanced to avoid possible side effects such as dental fluorosis or negative impact on tooth development [Esposti et al. 2021] [11].

Successive exposures to high concentrations of fluoride during tooth development can cause fluorosis, which is a disorder of tooth development that causes enamel with lower mineral content and increased porosity [Alvarez et al. 2009] [1]. As a consequence, there is a change in the surface texture, coloration (white to brown spots) and a decrease in enamel hardness, resulting in weakened teeth.

Another contraindication to oral hygiene products containing fluoride may be related to the use of dental implants or other metallic devices in the mouth. Studies show that the presence of fluorides can cause corrosion and release of metallic ions in the oral cavity [Schiff [32] et al. 2005; House [14] et al. 2008; Anwar[2] et al. 2011; Saporeti [30] et al. 2012; Yanisarapan [40] et al. 2018; Chen [6] et al. 2020].

Also, as an alternative to fluoride for caries prevention, a new approach has emerged in recent years with biomimetic agents (inspired by nature) that promote remineralization and inhibit demineralization of hard dental tissue. One such biomimetic agent is nanohydroxyapatite (nano-HAp), a calcium phosphate mineral – $Ca_5(PO_4)_3(OH)$. Nano-HAp is studied in different fields of preventive oral health. Unlike fluoride, accidental ingestion of nano-HAp as an ingredient in toothpaste is not associated with any relevant systemic health risk, as nano-HAp exists naturally as the major inorganic component of all human hard tissues, such as teeth and bones [Paszynska et al. 2021] [27].

Nano-HAp is one of the most studied biomaterials, given its proven biocompatibility and because it is the main constituent of the mineral part of bones and teeth. The biomimetic function of nano-HAp is to protect teeth by creating a new layer of synthetic enamel around the tooth, instead of just altering the surface layer as occurs with fluoride, which chemically transforms calcium halophosphate [$Ca_5(PO_4)_3F$] [Pepla et al. 2014] [28].

The main objective of the work, therefore, was to qualitatively evaluate the potential of nano-HAp in terms of its ability to remineralize and change the surface morphology of enamel and dentin, ex vivo, in three different formulations of toothpastes: pure synthetic nanohydroxyapatite, nano synthetic hydroxyapatite and fluoride and just fluoride. The specific objective was to analyze by scanning electron microscopy (SEM) and chemical analysis (EDS) the effectiveness of a new toothpaste without fluoride and with 5% nano-HAp in the mineral deposition of the tooth structure and obliteration of the dentinal tubules.

2 MATERIAL AND METHODS

This is a cross-sectional experimental study with a qualitative approach. The research was submitted and approved by the Univille Research Ethics Committee, under number 4,787,854.

2.1 SAMPLES

For the preparation of the samples, 4 mandibular third molars were selected, with a mean mesiodistal diameter of 19 mm on the crown. The teeth came from the Biobank of the Dentistry course at UNIVILLE.

2.2 PREPARATION OF SAMPLES

To make the samples, the dental crowns were sectioned into 4 parts with a diamond disc, totaling 16 samples in the experiment.

First, the teeth were positioned in PVC tubes (matrix) 18x21mm, vaselined, so that each sample was centralized and included in colorless acrylic resin (Jet Classic, São Paulo, SP, Brazil). The first cut was mesio-distally (Figure 1A), separating the tooth into two halves, one buccal and one lingual. To obtain the 2nd cut, each half of the tooth was again included in a Vaseline PVC cylinder. The embedded teeth were positioned horizontally on the cutting machine. The cuts in this step (Figure 1B) were made in the axial (transverse) direction. The first cut made was 1mm in order to remove only the cusp tips to obtain a completely smooth and uniform surface. The second cut was located in the middle third of the crown, in order to obtain two equal parts of the crown, with uniform exposure of dentin and enamel. At the end of these cuts, 4 samples were obtained from each tooth, with exposure of enamel and dentin (figure 2).

Figure 1: Schematic drawing of the cuts that were made on the teeth for sample preparation. In figure 1A (on the left) there is a representation of the first cut, performed in the mesio-distal direction. In figure 1B (on the right) there is a representation of the second cut, performed in the axial (transverse) direction.

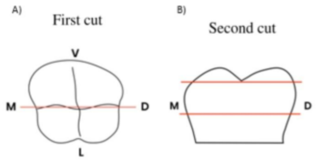
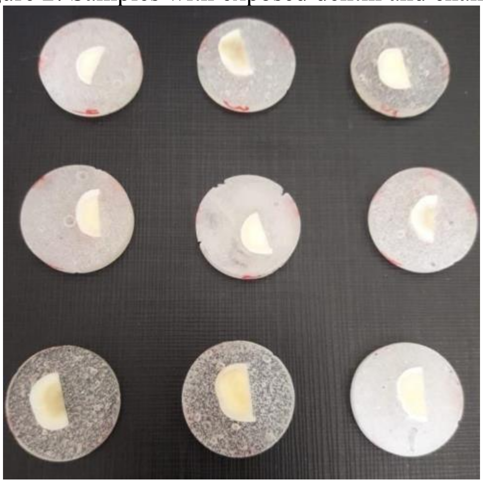


Figure 2: Samples with exposed dentin and enamel.



To smooth the surfaces and remove possible grooves, the samples were polished with 600 and 1200 granulation discs until they were smooth. After performing this step, the samples were properly cleaned by immersion in an ultrasonic tank with deionized water.

2.3 THE PROCESS OF DENTAL DEMINERALIZATION

To simulate dental demineralization, the cleaned samples were submitted to an acid challenge, in a 37% phosphoric acid solution (H_3PO_4) for 30 seconds and washed in abundant running water for 60 seconds.

2.4 DENTAL REMINERALIZATION PROCESS

Each sample was placed in a Petri disc, submerged in modified saline buffer solution (PBS). Samples in solution were stored in an oven at Certomat U (B. Braun, Germany) at 37 degrees Celsius, to resemble human body temperature. The samples were kept throughout the study inside the temperature-controlled machine, being removed only for brushing. All solutions used in this experiment were prepared at Univille's Biotechnology laboratory.

The samples were randomly divided into four groups for brushing, with a negative control (chart I).

Table I: Experimental groups

GROUPS	COMPOUND	BRAND
A	negative control	PBS
B	Toothpaste with Nano Hydroxyapatite 5% without fluoride	DNPRIME, Brazil - no trade name
W	Toothpaste with Nano Hydroxyapatite and Fluorine	Be You Yellow, Curaprox, Switzerland
D	fluoride toothpaste	Colgate Triple Action, Colgate, United States

The remineralization process was carried out by manual brushing, simulating the practice of toothbrushing. The method used to perform brushing with the 4 solutions was brushing each sample for 1 minute, twice a day, for 10 consecutive days. These brushings were performed with medium-sized disposable microbrushes, with about 1 ml of solution in each sample and washed after each brushing for 1 minute in running water. Brushing was carried out on the entire surface of the samples, both on dentin and enamel, to test enamel remineralization and obliteration of dentinal tubules.

After the last brushing, all samples were placed in a desiccator under vacuum overnight, to be sent for scanning electron microscopy (SEM) analysis.

2.5 QUALITATIVE ANALYSIS

The technique used for the qualitative analysis was the Scanning Electron Microscopy (SEM) (JEOL, JSM-6701F), to evaluate the mineral deposition on the surface of the sample and the obliteration of the dentinal tubules. Chemical analysis (EDS) was also used to identify nanohydroxyapatite depositions on the surface of the sample.

3 RESULTS

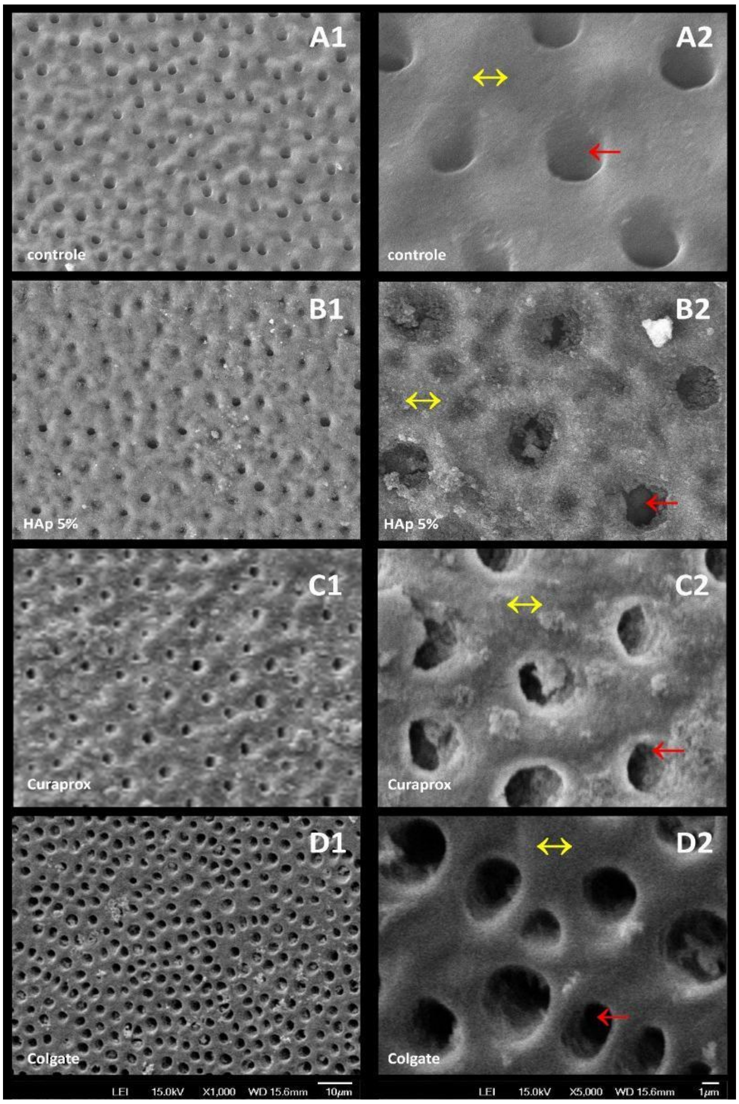
The analysis of mineral deposition on the surface of the samples and obliteration of dentinal tubules by Scanning Electron Microscopy (SEM) is shown in figure 1, with magnifications of 1000 and 5000x.

In the control group (figure 4 - images A1 and A2), it is observed that the dentinal tubules were not obliterated and the morphology of the dentin surface was not altered.

Figure 4 - images B1, B2, C1 and C2 (referring to the toothpaste with 5% nano-HAp without fluoride and the toothpaste with nano-HAp and fluoride) shows a mineral deposition on the surface of the dentin and on the inside the dentinal tubules, causing changes in the surface and morphology of the dentin, as well as the obliteration of the dentinal tubules. There is a difference in the appearance of the mineral deposition on the dentin surface between group B and group C, where in group B we can observe a more uniform deposition than in group C. At 5000x magnification (figure 4 - images B2 and

C2) it was identified the presence of nano-HAp within the dentinal tubules in these two groups (signaled by the red arrows), according to the result of the EDS analysis.

Figure 4: Images obtained by SEM with magnification of 1000x (left column) and 5000x (right column), referring to the different sample treatments. Images A1 and A2 refer to the control group. Images B1 and B2 refer to the fluoride-free 5% nano-HAp toothpaste (No trade name, DNPRIME, Brazil). Images C1 and C2 refer to the toothpaste with nano-HAp and fluoride (Be You Amarela, Curaprox, Switzerland). Images D1 and D2 refer to the fluoride toothpaste (Colgate Triple Action, Colgate, United States).



In figure 4 - images D1 and D2 (referring to the fluoride toothpaste), although it is also possible to visualize the deposition of mineral material, this occurred in a more subtle way and only within some dentinal tubules, not causing alteration in the morphology of the dentin.

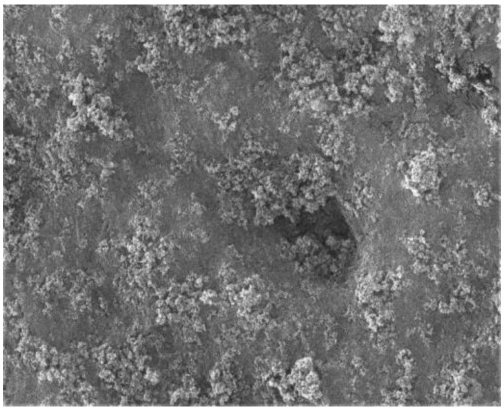
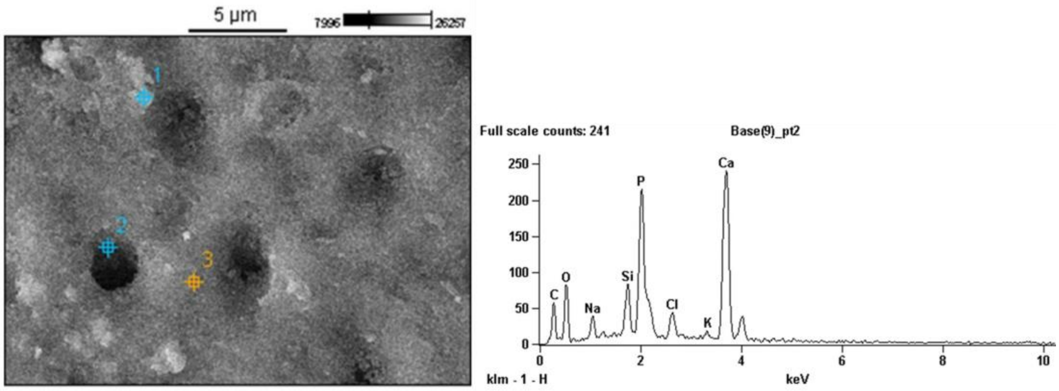


Figure 5: Image obtained by SEM, referring to a 10,000x magnification of S2 (figure 1). This image refers to the DNPRIME toothpaste sample, with 5% fluoride-free nano-HAp.

In Figure 5, it is possible to observe that the tubules are filled with material, this filling is uniform and from the “inside out”. It can be observed that the dentin is also filled with material in a homogeneous way, therefore, it can be said that there is a homogeneous interaction between the material and the dentin surface.

Figures 6 and 7: Image with 5000x magnification and graph referring to the EDS analysis of group B (toothpaste with 5% nano-HAp without fluoride).



In Figure 6, the image with a magnification of 5,000x of the sample in which toothpaste with 5% nano-HAp without fluoride was used, in which 3 points were randomly chosen (points 1 and 2 marked in blue and point 3 marked in yellow) for qualitative elemental analysis by EDS. The graph demonstrates the presence of phosphorus and calcium (main components of the nano-HAp), confirming the deposition of the nano-HAp on the dentin surface and within the dentinal tubules. The presence of sodium and phosphate is also observed, which may be residues of the modified saline buffer solution (PBS).

4 DISCUSSIONS

In 1970, the Japanese company Sangi Co Ltd was the first to become interested in hydroxyapatite, after buying the rights from NASA (National Aeronautics and Space Authority of the USA). Astronauts lost minerals from teeth and bones in weightlessness, and NASA proposed a synthetic hydroxyapatite as a repair material. The Sangi Co. Ltd came up with the idea in 1978 to launch toothpaste that could repair tooth enamel, which for the first time contains nano-HAp. In 2006, the first biomimetic toothpaste containing synthetic hydroxyapatite as an alternative to fluoride for the remineralization and repair of tooth enamel appeared in Europe.

Thus, the greatest contribution to the development of these biomimetic agents occurred with the advent of nanotechnology. Nano-HAp toothpaste (nano-HAp) toothpastes were first introduced and studied in Japan in the 1980s. Since then, several studies have reported greater or equal remineralization potential of nano-HAp toothpastes compared to conventional toothpastes containing fluoride, both in *in vitro studies* [Li [20] et al. 2008; Tschoppe [38] et al. 2011; Sadiasa [29] et al. 2013; Ebadifar [8] et al. 2017] and *in vivo* [Najibfard [22] et al. 2011; Bossù [5] et al. 2019; Badiee [4] et al. 2020 Paszynska [27] et al. 2021], and in vivo.

The aim of this study was to evaluate the potential of nano-HAp to remineralize tooth structure after acid challenge. Analyzing the results of the present study, it was observed that in the control group (Figures A1 and A2), the tubules were not obliterated and the morphology of the dentin surface was not altered, which was already expected, since no agent with remineralizing capacity was used. As for groups B (toothpaste with nano-HAp 5%) and C (toothpaste with nano-HAp and fluoride), it was possible to observe a mineral deposition on the surface of the dentin and inside the dentinal tubules, altering the morphology of the dentin, thus obliterating the dentinal tubules, which leads to a remineralization of the tooth structure. However, in this study, it was not possible to observe a synergistic effect when nano-HAp was associated with fluoride.

Currently, synthetic nano-HAp is accepted as a remineralizing agent, and its efficacy is widely described and validated in vitro and in vivo in the scientific literature. Different modes of nano-HAp activity in the oral cavity have already been established: (i) physical restoration, which is the fixation of nano-HAp particles to the tooth surface and cleaning properties; (ii) biochemical effect by releasing calcium and phosphate ions under acidic conditions forming an interface between nano-HAp particles and enamel; and (iii) biological interaction of nano-HAp particles with microorganisms. Furthermore, nano-HAp-based materials can desensitize exposed dentinal tubules, thus forming a mineralized barrier [Enax & Epple 2018] [9].

In figure 1D (fluoride toothpaste), although it is also possible to visualize the deposition of mineral material, evidencing a remineralization process, this occurred more subtly and only in the dentinal tubules, not causing a change in the morphology of the dentin. Similar results were also observed by Yuan [42] et al. (2012), the authors compared the use of a common dentifrice with a dentifrice added with nano-HAp (3%) in samples of discs of molar and premolar teeth, and also observed a change in the surface of the dentin and a reduction in the diameter of the teeth. dentinal tubules.

Najibfard[22] et al. (2011) performed a double-blind in situ study with thirty participants using enamel plates with and without artificial caries injuries. Participants brushed for at least 28 days with a fluoride toothpaste (1100 ppm fluoride) or a HAP toothpaste (5% HAP and 10% HAP, respectively). Plaques were analyzed by microradiography to quantify mineral loss and lesion depth. Results showed that HAP toothpaste prevented enamel demineralization.

One of the first studies into the potential of nano HAp as a remineralizing agent demonstrated that synthetic nanometer hydroxyapatite (nano-HAp) can rebuild human enamel without prior excavation, in a process that not only repairs early caries lesions, but also helps to prevent its recurrence, strengthening the natural enamel [Yamagishi et al. 2005] [39.] Similar results were also observed in bovine teeth [Huang et al. 2009] [15,] in which they not only verified the remineralization process, but also demonstrated the ability of nano-HAp to significantly increase surface microhardness.

The effect of nano-HAp on the *in vitro remineralization* of bovine enamel and dentin was evaluated by microradiography [Tschoppe et al. 2011] [38.] Nano-HAp toothpastes demonstrated greater dentin remineralization capacity and similar enamel remineralization capacity when compared to fluoride dentifrices.

In this study, dentin remineralization was evident with the use of nano-HAp. However, as it is an in vitro study, the samples were only subjected to a single acid challenge, which provided controlled demineralization. This situation does not occur in the oral cavity, where the process of demineralization and remineralization is constant. From this premise, clinical studies are recommended, with patients, in order to prove and deepen these results. Another important issue, observed from this study, which evidenced the obliteration of dentinal tubules, is the suggestion of studies on the use of nano-HAp in dentin sensitivity, which today affects a significant portion of the population and constitutes a challenge in relation to the treatment of dental surgeon. From the results, it is believed that products with nano-HAp may have promising results in dentin desensitization, whose main cause is the exposure of dentinal tubules.

5 FINAL CONSIDERATIONS

There is a growing demand for biomimetic materials in oral care products. Also, it was seen that nano-HAp has promising results to promote remineralization of tooth structure. The resemblance of nano-HAp to the enamel and dentin of natural teeth has been a focus in recent years. Therefore, its non-toxic and biomimetic property offers an advantage over conventional products.

From the results obtained in this study, through scanning electron microscopy (SEM) and chemical analysis (EDS), it can be concluded that nano-HAp has remineralizing potential, proving the effectiveness of a new toothpaste without fluoride and with 5% nano-HAp in mineral deposition of tooth structure and obliteration of dentinal tubules.

REFERENCES

ALVAREZ, JA; REZENDE, M; SALAZAR, M; ALVES, F; CELIBERTI, P; CIAMPONI, L. Dental fluorosis: Exposure, prevention and management. Oral Medicine, Oral Pathology and Oral Surgery, v. 14, no. 2, 2009.

ANWAR, EM; KHEIRALLA, LS; TAMMAM, RH Effect of fluoride on the corrosion behavior of Ti and Ti6Al4V dental implants coupled with different superstructures. Journal of Oral Implantology, v. 37, no. 3, p. 309–317, 2011.

ASPINALL, SR; PARKER, JK; KHUTORYANSKIY, VV Oral care product formulations, properties and challenges. Colloids and Surfaces B: Biointerfaces, v. 200, no. October 2020, p. 111567, 2021.

BADIEE, M.; NIMA, J; SAYEDALI, F; NAZILA, A; SHAHIN, K; ASGHAR, E. Comparison of the effects of toothpastes containing nanohydroxyapatite and fluoride on white spot lesions in orthodontic patients: A randomized clinical trial. Dental Research Journal, v. 17, no. 5, p. 354–359, 2020.

BOSSÙ, M.; SACCUCCI, M; SALUCCI, A; GIANNI, G; BRUNI, E; UCELLETTI, D. et al. Enamel remineralization and repair results of Biomimetic Hydroxyapatite toothpaste on deciduous teeth: An effective option to fluoride toothpaste. Journal of Nanobiotechnology, v. 17, no. 1, p. 1–13, 2019.

CHEN, WQ; ZHANG, SM; QIU, J. Surface analysis and corrosion behavior of pure titanium under fluoride exposure. Journal of Prosthetic Dentistry, v. 124, no. 2, p. 239.e1-239.e8, 2020.

DO, LG Guidelines for use of fluorides in Australia: update 2019. Australian Dental Journal, v. 65, no. 1, p. 30–38, 2020.

EBADIFAR, A.; NOMANI, M.; FATEMI, SA Effect of nano-hydroxyapatite toothpaste on microhardness of artificial carious lesions created on extracted teeth. Journal of Dental Research, Dental Clinics, Dental Prospects, v. 11, no. 1, p. 14–17, 2017.

ENAX, J.; EPPLE, M. Synthetic hydroxyapatite as a biomimetic oral care agent. Oral health & preventive dentistry, v. 16, no. 1, p. 7–19, 2018.

ENAX, J.; FABRITIUS, H; VILPOUX, K; AMAECHI, B; MEYER, F.. Modes of Action and Clinical Efficacy of Particulate Hydroxyapatite in Preventive Oral Health Care − State of the Art.The Open Dentistry Journal, v. 13, no. 1, p. 274–287, 2019.

ESPOTI, LD; LONESCU, A; BRAMBILLA, E; TAMPIERI, A; LAFISCO, M. Characterization of a toothpaste containing bioactive hydroxyapatites and in vitro evaluation of its efficacy to remineralize enamel and to occlude dentinal tubules. Materials, v. 13, no. 13, p. 1–13, 2020.

FABRITIUS-VILPOUX, K.; ENAX, D; HERBIG, M; DIERK, I. Quantitative affinity parameters of synthetic hydroxyapatite and enamel surfaces in vitro. Bioinspired, Biomimetic and Nanobiomaterials, v. 8, no. 2, p. 141–153, 2018.

FABRITIUS-VILPOUX, K.; ENAX, D; HERBIG, M; DIERK, I. Quantitative affinity parameters of synthetic hydroxyapatite and enamel surfaces in vitro. Bioinspired, Biomimetic and Nanobiomaterials, v. 8, no. 2, p. 141–153, 2018.

HOUSE, K.; SERNETZ, F; DYMOCK, D; SANDY, J; IRELANDA, A. Corrosion of orthodontic appliances-should we care? American Journal of Orthodontics and Dentofacial Orthopedics, v. 133, no. 4, p. 584–592, 2008.

HUANG, SB; GAO, SS; YU, HY Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomedical Materials, v. 4, no. 3, p. 0–5, 2009.

IONESCU, AC; CAZZANIGA, G; OTTOBELLI, M; GODOY, F; BRAMBILLA, E. Substituted nano-hydroxyapatite toothpastes reduce biofilm formation on enamel and resin-based composite surfaces. Journal of Functional Biomaterials, v. 11, no. 2, 2020.

JIN, J.; XU, X; LAY, G; KUNZELMANN, K. Efficacy of tooth whitening with different calcium phosphate-based formulations. European Journal of Oral Sciences, v. 121, no. 4, p. 382–388, 2013.

KÖRNER, P.; SCHLEICH, J; WIEDEMEIER, D; ATTIN, T; WEGEHAUPT, F. Effects of Additional Use of Bioactive Glasses or a Hydroxyapatite Toothpaste on Remineralization of Artificial Lesions in vitro. Caries Research, v. 54, no. 4, p. 336–342, 2020.

LEE, BS; CHOU, P; CHEN, S; LIAO, H; CHANG, C. Prevention of enamel demineralization with a novel fluoride strip: Enamel surface composition and depth profile. Scientific Reports, v. 5, no. 1, p. 1–10, 2015.

LI, L; PAN, H; TAO, J; XU, X; MAO, C; GU, X; TANG, R. Repair of enamel by using hydroaxyapatite nanoparticles as the building blocks. Journal of Materials Chemistry, v. 18, no. 34, p. 4079–4084, 2008.

LIM, J.-H.; BAIRI, V; LINDER, S; FONG, A. Detection and Characterization of Silver Nanostructures in Consumer Products. Journal of Nanoscience and Nanotechnology, v. 19, no. 12, p. 8078–8087, 2019.

NAJIBFARD, K.; RAMALINGAN, K; CHEDJIEU, I; AMAECHI, B. Remineralization of early caries by a nano-hydroxyapatite dentifrice. Journal of Clinical Dentistry, v. 22, no. 5, p. 139–143, 2011.

OLEK, A.; KLIMEK, L.; BOTTACZ-RZEPKOWSKA, E. Comparative scanning electron microscope analysis of the enamel of permanent human, bovine and porcine teeth. Journal of Veterinary Science, v. 21, no. 6, p. 1–14, 2020.

ORILISI, G.; TOSCO, V; MONTERUBBIANESI, R; NOTARSTEFANO, V; OZCAN, M; PUTIGNANO, A; et al. ATR-FTIR, EDS and SEM evaluations of enamel structure after treatment with hydrogen peroxide bleaching agents loaded with nano-hydroxyapatite particles. PeerJ, v. 9, p. 1–24, 2021.

ORSINI, G.; PROCACCINI, M; MANZOLI, L; GIULIODORI, M; LORENZINI, A; PUTIGNANO, A. A double-blind randomized-controlled trial comparing the desensitizing efficacy of a new dentifrice containing carbonate/hydroxyapatite nanocrystals and a sodium fluoride/potassium nitrate dentifrice. Journal of Clinical Periodontology, v. 37, no. 6, p. 510–517, 2010.

OXIDE, S. DE; ESTANHO, D. Research Report Research Report. n. January, p. 1–51, 2017.

PASZYNSKA, E.; PAWINSKA, M; GAWRIOLEK, M; KAMINSKA, I; OTULAKOWSKA, J; KOLADA G. et al. Impact of a toothpaste with microcrystalline hydroxyapatite on the occurrence of early childhood caries: a 1-year randomized clinical trial. Scientific Reports, v. 11, no. 1, p. 1–15, 2021.

PEPLA, E. Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: a review of literature. Annali di Stomatologia, p. 108–114, 2014.

SADIASA, A.; DONG-WHOO, J; NATH, S; SEO, H; LEE, H. Addition of Hydroxyapatite to Toothpaste and Its Effect to Dentin Remineralization. Korean Journal of Materials Research, v. 23, no. 3, p. 168–176, 2013.

SAPORETI, MP; MAZZIEIRO, ET; SALES, WF In vitro corrosion of metallic orthodontic brackets: Influence of artificial saliva with and without fluorides. Dental Press Journal of Orthodontics, v. 17, no. 6, p. 1–7, 2012.

SAREMBE, S.; ENAX, J; MORAWIETZ, M; KIESOW, A; MEYER, F. In Vitro Whitening Effect of a Hydroxyapatite-Based Oral Care Gel. European Journal of Dentistry, vol. 14, no. 3, p. 335–341, 2020.

SCHIFF, N.; GROSGOGEAT, B; LISSAC, M; DALARD, F. Influence of fluoride content and pH on the corrosion resistance of titanium and its alloys. Biomaterials, v. 23, no. 9, p. 1995–2002, 2002.

SCHUNEMANN, WVH Effect of metallic particles released from dental implants on peri-implant tissues TT - The effect of metallic particles released from dental implants on peri-implant tissues. ImplantNewsPerio, v. 1, no. 4, p. 701–709, 2016.

SOARES, CJ; PIZI, E; FONSECA, R; MARTINS, L. Influence of root embedment material and periodontal ligament simulation on fracture resistance tests. Brazilian dental research = Brazilian oral research, v. 19, no. 1, p. 11–16, 2005.

STEINERT, S.; KUCHENBECKER, J; MEYER, F; SIMADER, B; ZWANZIG, K; ENAX, J. Whitening effects of a novel oral care gel with biomimetic hydroxyapatite: A 4-week observational pilot study. Biomimetics, v. 5, no. 4, p. 1–9, 2020.

STEINERT, S.; ZWANZIG, K; DOENGES, H; KUCHENBECKER, J; MEYER, F; ENAX, J. Daily application of a toothpaste with biomimetic hydroxyapatite and its subjective impact on dentin hypersensitivity, tooth smoothness, tooth whitening, gum bleeding, and feeling of Freshness. Biomimetics, v. 5, no. 2, p. 1–11, 2020.

SUDRADJAT, H.; MEYER, F; LOZA, K; EPPLE, M; ENAX, J. In Vivo Effects of a Hydroxyapatite-Based Oral Care Gel on the Calcium and Phosphorus Levels of Dental Plaque. European Journal of Dentistry, vol. 14, no. 2, p. 206–211, 2020.

TSCHOPPE, P.; ZANDIM, D; MARTUS, P; KIELBASSA, A. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. Journal of Dentistry, vol. 39, no. 6, p. 430–437, 2011.

YAMAGISHI, K.; ONUMA, K; SUZUKI, T; OKADA, F; TAGAMI, J; OTSUKI, M. et al. A synthetic enamel for rapid tooth repair. Nature, v. 433, no. 7028, p. 819, 2005.

YANISARAPAN, T.; THUNYAKITPISAL, P.; CHANTARAWARATIT, P. ON. Corrosion of metal orthodontic brackets and archwires caused by fluoride-containing products: Cytotoxicity, metal ion release and surface roughness. Orthodontic Waves, v. 77, no. 2, p. 79–89, 2018.

YUAN, P.; SHEN X; LIU J; HOU Y; ZHU M; HUANG J; XU P. Effects of Dentifrice Containing Hydroxyapatite on Dentinal Tubule Occlusion and Aqueous Hexavalent Chromium Cations Sorption: A Preliminary Study. PLOS ONE, December 2012 | Volume 7 | Issue 12 | e45283.

YOUSSEF, A.; FARID, M; ZAYED, M; LYNCH, E; ALAM, M; KIELBASSA, A. Improving oral health: a short-term split-mouth randomized clinical trial revealing the superiority of resin infiltration over remineralization of white spot lesions. Quintessence international (Berlin, Germany: 1985), v. 51, no. 9, p. 696–709, 2020.

ZANDIM, DL; TSCHOPPE, P; SAMPAIO, J; KIELBASSA, A. Effect of saliva substitutes in combination with fluorides on remineralization of subsurface dentin lesions. Supportive Care in Cancer, v. 19, no. 8, p. 1143–1149, 2011.