International Summit on Science Technology and Humanity
ISETH 2023
ISSN: 2807-7245 (online)

Effectiveness of Nano-hydroxyapatite in Caries Prevention: Literature Review

Tri Sulistyawati[1*], Cahyani[2]

[1]*Student, Faculty of Dentistry, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia*
[2]*Lecturer, Faculty of Dentistry, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia*

Abstract

Introduction: Enamel, as the most resilient biological tissue found in the human organism, comprises an estimated 96% inorganic matter. The principal constituent, which confers upon enamel its characteristic translucency and pristine white appearance, is Hydroxyapatite (HA). Ithe realm of dentistry, the emergence of nanohydroxyapatie (nHA) has garnered noteworthy recognotion owing to its exceptional hydroxyapatite. Dentistry has witnessed the assimilation of nHA into diverse dental formulations and materials, thereby fostering comprehensive oral healthcare practices. Notably, a prominent utilization of nHA lies in its role in the prevention of dental caries, leading to development of remineralization pastes. These products aim to enhance remineralization and protect the tooth surface. Additionally, nHA based materials have shown promise in restoring dental defects such as caries, fractures and tooth loss. Overall, nHA holds potential for advancing preventive and restorative dentistry. **Objectives:** The objective of this literature review is to assess the effectiveness of nano-hydroxyapatite in preventing dental caries. **Methods:** A thorough review of pertinent research, studies, and publications will beundertaken, specifically examining the role nHA in preventing dental caried. The chosen articles will undergo critical evaluation to assess the overall efficacy of nHA in caries prevention. **Result**: The deposition of mineral on carious lesions can be facilitated by the remarkable capacity of nanohydroxyapatite. Through its chemical affinity with apatite crystals, nanohydroxyapatite aids in the remineralization of enamel caries by generating a uniform apatite layer on the demineralized surface of the enamel. **Conclusions:** Based on this comprehensive analysis of existing literature, it can be inffered that nanohydroxyapatite (nHAP) exhibits the potential to stimulate mineralization on incipient carious lesions by effectively permeating the porous tooth structure resulting from the caries process. Consequently, this infiltration enhances the mineral content and hardness of the affected tooth structure.

Keywords: Demineralization, Hydroxyapatite, Dental Caries, Nanohydroxyapatite, Remineralization

Introduction

The biocompatibility and prevalence of hydroxyapatite (HA) as a major constituent in the rigid tissues of the human body, such as bones and teeth, have positioned it as a subject of profound interest within the ralms of medicine and dentistry. Nano-hydroxyapatite (nano-HAp), with its similar size, crystallography, and chemical composition to natural hard tissues, has emerged as a promising biomaterial for various dental applications (Pushpalatha *et al.,* 2023). One of its potential uses is in the prevention of dental caries, although the controversies surrounding its efficacy and safety warrant further exploration.

Compared to conventional HA, nano-HAp possesses several advantages, including improved solubility, enhanced bioactivity, and better biocompatibility. These benefits are attributed to its larger surface area, which allows for increased interactions with the oral environment. Thus, nano-hydroxyapatite (nano-HAp) has been effectively utilized in various

[1*]Corresponding author: j520180081@student.ums.ac.id

domains of dentistry, including implantology, management of dentin hypersensitivity, bleaching procedures, as well as prevention and restoration of dental caries. Although the application of nanotechnology in dentistry has been extesively explored, concerns persist regarding the cytotoxicity and safety of nano-HAp. Research studies indicate that nano-HAp particles have the potential to traverse the oral epithelium and enter the systemic circulation, thereby potentially inducing cytotoxic effects. In light of the imperative for safe materials in oral healthcare, it is of utmost importance to conduct comprehensive evaluations to ascertain the potential risks associated with the utilization of nano-HAp (Pushpalatha *et al*., 2023 ; Izzetti R *et al*., 2022).

One area of interest is the use of nano-hydroxyapatite in remineralizing caries-affected enamel and dentin. In early caries lesions, mineral ions are lost due to acidic byproducts from bacteria, while the collagen matrix remains intact. Nano-HAp can directly remineralize the organic structure by replacing the lost minerals. Incorporating nano-HAp into toothpaste formulations allows for its application to the tooth surface, where the nanoparticles penetrate the porous enamel to form a protective film. In vitro studies have demonstrated that nano-HAp toothpaste can reduce caries development and increase enamel hardness. Additionally, the combination of nano-HAp with mouth rinses containing sodium fluoride has shown a synergistic effect on caries remineralization in enamel. The topical application of nano-HAp has also shown promising results in preventing caries in the oral cavity (Najibfard *et al*., 2021;Anil *et al.*, 2022;).

Nano-hydroxyapatite is widely recognized for its role in preventing dental caries by fostering the remineralization of enamel and dentin affected by caries. In the inital phases of caries, the hard tissues of the teeth undergo mineral ion loss due to acidic byproducts from bacteria, while the collagen matrix remains unaffected. Nano-hydroxyapatite effectively restores the lost minerals, facilitating the remineralization the organic structure. One strategy to achieve this outcome involves integrating nano-hydroxyapatite (nHA) into toothpaste formulation. Upon application to the tooth surface, synthesized nHA nanoparticles enter the tooth's porosity, establishing a protective film. A laboratory investigation into the impact of nHA toothpaste on remineralization revealed its ability to effectively diminish caries development and enhance enamel hardness. Moreover, when used alongside mouth rinses containing sodium fluoride, nano-HA displayed a synergistic effect in remineralizing caries in enamel. Additionally, the directly topical application of nano-HA in the oral cavity has shown potential in caries prevention (Anil *et al.*, 2022; Grocholewicz *et al.*, 2020; Wierichs *et al.*, 2022) However, it is important to consider that the use of nano-hydroxyapatite in caries prevention is a topic of controversy and ongoing research. This literature review aims to examine the effectiveness of nano-hydroxyapatite in preventing dental caries, taking into account the various perspectives and debates surrounding its application.

Literature Review

Dental caries

Dental caries, colloquially referred to as tooth decay or cavities, represents a complex pathological condition that arises when the dental hard tissues, incluiding enamel, dentin, and cementum, undergo degradation as a result of acidogenic bacteria found within dental plaque. The progression of caries encompasses a dynamic interplay between demineralization and remineralization processes. Upon metabolizing sugars and fermentable carbohydrates, the bacteria within dental plaque generate acidic byproducts, most notably lactic and acetic acid. These acids precipitate a reduction in the pH of the oral milieu, thereby instigating (Amaechi *et al*., 2019). Meanwhile, remineralization is the natural repair process that occurs when the pH in the oral environment returns to a more neutral level. Saliva plays a crucial role in this process by providing calcium, phosphate, and fluoride ions that help rebuild the mineral structure of the tooth. The balance between demineralization and remineralization is influenced by various factors, including oral hygiene practices, diet, saliva composition, and fluoride exposure. Effective oral hygiene, a balanced diet low in fermentable carbohydrates, and regular fluoride use can enhance remineralization and prevent or slow down the progression of dental caries (Imran *et al*., 2023).

Nano-hydroxyapatite synthesis

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is an inorganic mineral containing mainly calcium and phosphate with a Ca/P ratio of 1.67 (stoichiometric) (Figure 1). Hydroxyapatite can be chemically synthesized or derived from natural sources. The synthesis of nano hydroxyapatite can be achieved through various methodologies, incluiding co-precipitation, wet deposition, hydrothermal, mechanochemical, hydrolysis, solid state,and sol-gel methods. Among these approaches, the wet chemical precipitation method stands out as the most commonly employed technique due to its widespread reproducibility, simplicity, and cost-effectiveness. Nano-hydroxyapatite derived from the wet precipitation method typically exhibits nonstoichiometric characteristics, whereas the solid state method yields stoichiometric nHA. For large-scale and swift synthesis of nHA powder, the microwave hydrothermal method coupled with ultrasonic atomization precipitation can be employed. This particular method yields nanopowders with uniform size distribution, showcasing exceptional quality (Cai *et al.,* 2019). The attainment of nucleation control and crystal stability during the syinthesis process can be facilitated through the utilization of precipitants such as citrate, amino acidsm or EDTA (*ethylenediaminetetraacetic acid).* Furthermore, electrospinning techniques offer a viable means of obtaining nano-hydroxyapatite fibers (Fauziyah *et al*., 2021).

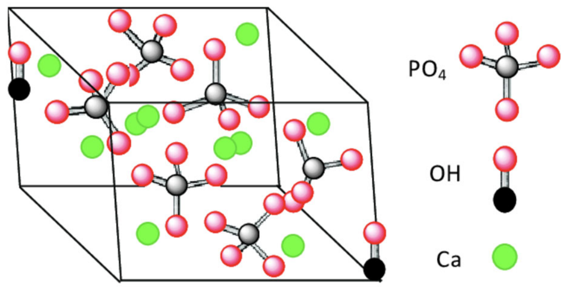

Figure 1 : Structure of Hydroxyapatite (Rujitanapanich *et al.,* 2014)

The role of nHAP in enamel remineralization

Nano-hydroxyapatite, akin to the naturally occurring hydroxyapatite crystals found within teeth, represents a biocompatible synthetic material that exhibit the propensity to foster remineralization, particularly within acidic enviroments, through the augmentation of calcium and phosphorus ions within the demineralization zone. Extensive research has examine dits therapeutic potential in tooth remineralization. The underlying principle behind the application of nano-hydroxyapatite lies in the regulation of the demineralization-remineralization equilinrium through salivary saturation of apatite minerals, elevated level of salivary calcium, and increased phosphate concentrations, which collectively promote reminelazation in teeth while contomitantly inhibiting demineralization. Notably, nano-hydroxyapatite has demonstrated the ability to enhace surface energy, augment atomicity, and establish robust bonding with the enamel surface (Imran *et al*., 2023).

In the study conducted by Najibfard *et al* (2021) the evaluation of enamel remineralization was conducted following the application of toothpaste containing different concentrations of nano-hydroxyapatite (nano-HAp) (5% or 10% nano-HAp) in comparison to toothpaste with 1100 ppm fluoride and 10% nano-HAP. It is worth noting that no statistically significant differences were observed between the two groups in terms of mineral loss and the depth of carious lesion penetration. Nevertheless, all formulations of toothpaste demonstrated a significant increase in mineral content on the surface of enamel lesions.

Wierichs *et al* (2020) the objective of the study was to assess the impact of four distinct toothpaste formulations on the process of remineralization. The experimental group utilized toothpaste incorporating a blend of zinc-carbonate and hydroxyapatite nanocrystals, with varying fluoride concentrations (1100 or 5000 ppm F), whereas the control group employed flouride-free toothpaste, comprising nano-HAp, did not exhibit significant efficacy in impeding demineralization.

Grocholewicz *et al* (2020) the aim of the study was to assess the process of enamel remineralization in the context of early carious lesions following treatment with nano-hydroxyapatite (nano-HAp) gel, gaseous ozone therapy, or a combined approach involving both treatments. The authors arrived at the conclusion taht the combined administration of nano-HAp gel and ozone therapy yielded the most favorable outcome when compared teh individual utilizationof either nano-HAp or ozone therapy alone.

The role of nHAp in enamel demineralization

Nano-hydroxyapatite (nHAp) offers a vaible calcium source for the oral cavity, whereby elevated calcium leveles contribute to acid inhibition, demineralization reduction, and enhanced enamel remineralization. Multiple studies have expounded upon the ability of nHAp to diminish caries susceptibility, amplify enamel remineralization, impede caries development, and mitigate demineralization. This phenomenon can be attributed to the formation of a novel, uniform apatite surface layer on demineralized surfaces, which serves as a protective barrier against further demineralization.

Moreovernano-hydroxyapatite facilitates the accumulation of minerals within the outer layer of carious lesions, thereby inducing a heightened degree of mineralization that effectively hinders the infiltration of mineral ionsinto the deeper regions of the demineralized lesion (Izzetti R *et al.,* 2022).

Research Methods

Data Search

The initial search was conducted from October to November 2023 through 5 database : (1) Pubmed, (2) Taylor & Francis, (3) ScienceDirect, (4) Springer, (5) MDPI. The keywords in this search process are *nano-hydroxyapatite, nanotechnology, hydroxyapatites, operative dentistry, dental caries, remineralization, demineralization.*

Inclusion Criteria

a. Articles published within the last 5 years
b. Articles can be accessed in *full text* and in English
c. Articles that discuss the use of *nano-hydroxyapatite* in the prevention of dental caries
d. Articles that discuss the effects of *nano-hydroxyapatite* in the prevention of dental caries
e. Articles discussing the effects of nHAP mineralization on dental hard tissues (including remineralization and modulation of demineralization)

Exclusion Criteria

a. Articles in abstract form or not accessible in *full text*
b. Articles published more than 5 years ago
c. Articles that are not in english
d. Articles discussing the use of *nano-hydroxyapatite* in general medicine

Table 1. Descriptive Characteristics of Articles

No	Journal Author	Tahun	Location
1.	(Izzetti R *et al.,* 2022)	2022	Italy
2.	(Pushpalatha *et al.,* 2023)	2023	India
3.	(Imran *et al.,* 2023)	2023	Pakistan
4.	(Wierichs *et al.,* 2022)	2022	Switzerland
5.	(Juntavee A *et al.,* 2021)	2021	Thailand

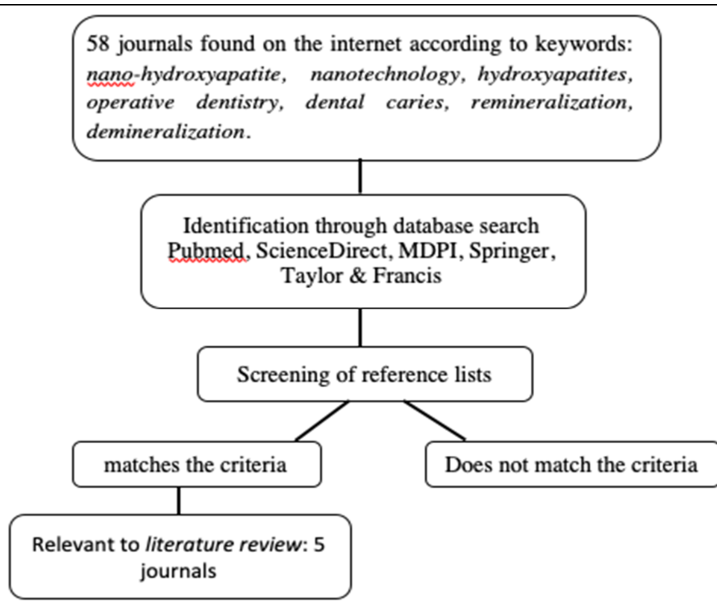

Figure 2. Flow diagram map

Results

Five articles that became relevant journals were published within the last 5 years and used English. There were 1 article published in 2021, 2 articles published in 2022, and 2 articles published in 2023. Articles were obtained through search on Pubmed, Taylor & Francis, ScienceDirect, Springer and MDPI.

First article

The findings elucidated that nano-hydroxyapatite (nano-HAp) holds a prominent position as a frequently employed biomateral within the realm of dentistry, owing to its osteogenic properties and biocompatibility. A comprehensive literature search was conductrd via reputable databases such as PubMed adn scopus, yielding a total of 154 articles. Through a meticulous analysis of titles, absracts, and full-text readings, a subset of 26 articles met the predefined inclusion criteria. Among these, three studies expounded upon the potential of nano-HAp for tissue remineralization, while eight studies highlighted its efficacy in the treatment of dentin hypersensitivity. Additionally, two studies investigated the role of nano-HAp in the field of orthodontics, whereas 13 studies evaluated its impact within the domains of periodontology and implantology. Drawing upon the collective findings of these studies, it can be concluded that nano-HAp exhibits effectiveness in caries prevention, serves as a desensitizing agent for dentin hypersensitivity treament, and demonstrates utility as a bone graft materials/

Second article

Nano-hydroxyapatite (nHA) has found wide-ranging application in multiple domains of dentistry, including implantology, surgery, periodontology, aesthetics, and preventive dentistry. In comparison to conventional hydroxyapatite (HA), nHA exhibits enhaced properties such as increased solubility, higher surface energy, and improved biocompatibilty. These advantageous attributes arise from the morpholgical and structural similarities between nano-sized hydroxyapatite particles and dental hydroxyapatite crystal. Consequently, these nanoparticles have been sucessfully integrated into various dental formulations, serving diverse purposes in comprehensive oral healthcare. Notably, nHA-based products such as toothpastes, mouth rinses, and remineralization pastes have been developed specifically for dental caries prevention, ensuring a comprehensive approach to otal health maintenance.

Third article

A literature search was conducted in PubMeD by searching for journals published in 2010-2022. In total, 1052 articles were found. After analyzing the titles, abstracts, and reading the full text, only 38 articles met the inclusion criteria. Among these articles, 29 studies analyzed the effect of nHAP on enamel mineralization, 5 studies analyzed dentin remineralization and 1 study on cementum and 3 studies investigated the antimicrobial effect of nHAP on cariogenic bacteria. The findings indicated that nano-hydroxyapatite (nHAp) exhibits the capacity to facilitate mineralization in the initial stage of caries development. It achieves this by permeating the porous tooth structure thereby augmenting its mineral content and hardness. The particle size of nHAp assumes a crucial role in the process of remineralization. Additionally, the incorporation of metallic substitutes within the nHAp structure enables it to exhibit antimicrobial properties. Collectively, these attributes position nHAp as a highly promising bioactive material for effective dental caries management.

Fourth article

This comprehensive review systematically analyzed in vivo or in situ investigations focusing on the effectiveness of nanohydroxyapatite (nHAP) in etiher remineralizing early caries lesions or preventing the occurrence of caries. The articles were collected through thorough searches of Central Cochrane, PubMed-MEDLINE, and Ovid EMBASE databases. A total of five in vivo studies an five in situ studies, meeting the predeterminerd inclusion criteria and involving at least 633 teeth (10131 specimen), were identified. Due to significant heterogeneity in study designs and variations in outcomes, it was not feasible to conduct a meta-analysis for the in vivo studies. The in situ studies indicated that NaF demonstrated the capability to inhibit demineralization, while nHAP did not yield comparable results. However, under remineralization conditions, both nHAP and NaF exhibited similar effects. It is essential to emphasize that the level of evidence supporting these findings was determined to be very low. Moreover, six studies exhibited a high risk of bias, and an additional six studies were identified as being funded or published by the manufacturers of these products.

Fifth article

In this investigation, the effects of two distinct concentrations of *nano-hydroxyapatite gel* (NHG) on the remineralization of simulated caries lesions were assessed in comparison to nano-HA toothpaste (NHT) and *fluoride varnish* (FV). The results indicated that NHT exhibited a significantly greater capacity for remineralization compared to both NHG and FV ($p < 0.05$). Notably, both toothpastes and gel formulations containeing nano-HA demonstrated superior remineralization potential when compared to fluoride varnish. NHG, specifically at concentrations of 20% and 30%, emerged as a recommended remineralizing agent capable of effectively addressing caries lesions. The study highlights the efficacy and non-toxic natur of nano-hydroxyapatite gel, rendering it a suitable option for uncooperative peridiatric patients or individuals experiencing discomfort when employing traditonal oral hygiene measures such as toothbrushes and toothpaste for oral health maintenance.

Discussion

In recent years, the utilization of nano-hydroxyapatite (nHAp) has garnered significant attention as a preventive, therapeutic, and regenerative therapy. Hydroxyapatite (HA), a mineral widely employed in periodontal bone regenaration, dentin hypersensitivity treatment, and caries prevention as a remineralizing agent, has been the focus of considerable research. The primary constituent of tooth enamel comprises HA crystals ranging in size from 20 to 40 mm. Synthetic nano-hydroxyapatite has emerged as a promising candidate for remineralization purposes due to its structural and chemical resemblance to enamel apatite crystaks on a nanoscale. Furthermore, nano-hydroxyapatite exhibits heightened biocompatibility, robust bioactivity, resoprtion capabilities, and mechanical properties compared to HA. Experimental studies have demonstrated the remineralizing effects of nano-hydroxyapatite on artificial caires lesions and its capacity to regenerate enamel (Izzetti *R et al.,* 2022).

The utilization of diverse nano-hyroxyapatite (nano-HAp) formulations has gained widespread recognition in promoting enamel remineralization and preventing dental caries. However, the outcomes obtained from employing nano-HAp toothpaste appear comparable to those achieved with fluoride toothpaste. Consequently, it is rasonable to assert that nano-HAp based toothpastes can be safely incorporated as an adiditonal measuer in caries prevention strategiesm while also mitigating the risk of fluorosis (Izzetti R *et al.,* 2022). Meanwhile, grocholewicz *et al* (2020) conducted a study that combined nano-HAp with ozone therapy. Ozone therapy has demonstrated efficacy in managing early carious lesions,a lthough there is presently insufficient reliable evidence to support the notion that the application of ozone gas to carious tooth surfaces can arrest or reverse the progression of caries. Hence, it can be inferref that there is inadequate substantiation to recommend the combined treatment involving nano-HAp gel and ozone therapy.

Since 1980, the incorporation of nano-hydroxyapatite in toothpaste for caries prevention has gained prominence, particularly in Japan. Notably, in 1993, the Japanese Government officially approved nano-hydroxyapatite as an anti-caire agent. The preventive efficacy of nano-hydroxyapatite against dental caries stems from its robust binding affinity with proteins, plaque fragments, and bacteria, thereby augmenting the available surface area for protein binding. According to certain researchers, the underlying mechanism of nanohydroxyapatite involves facilitating remineralization through the deposition of apatite nanoparticles onto enamel defects. Additionally, nano-hydroxyapatite functions as a source of calcium phosphate, thereby sustaining supersaturation condtions within enamel minerals, which ultimately enhances remineralization and impedes demineralization processes (Philip N *et al*., 2019).

Nano-hydroxyapatite possesses the capacity to facilitate mineral deposition on carious lesions, owing to its surface bioactivity and chemical and physical resemblance to tooth enamel. Through a chemical bonding process with apatite crystals, nanohydroxyapatite aids in the remineralization of enamel caries by generating a uniform apatite layer on the demineralized enamel surface. Given the highly porous nature of early caries lesions, the penetration of nano-hydroxyapatite is significantly enhanced. Consequently, these nanoparticles serve as templates, attracting calcium and phosphate ions, thereby promoting the growth of apatite crystals (Figure 3) (Pushpalatha *et al*., 2023).

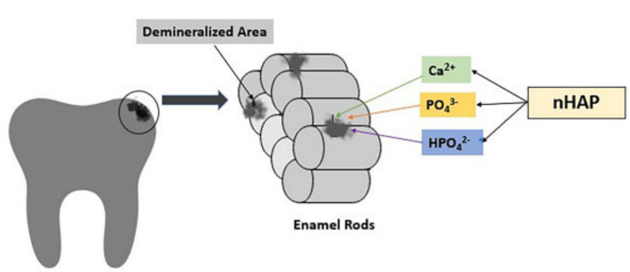

Figure 3. Mechanism of action of nanohydroxyapatite (nHAP) on early enamel caries (Pushpalatha et al., 2023)

According to the findings of Huang *et al* (2021), the optimal concentration for remineralizing early caries lesions was determined to be a 10% nano-hydroxyapatite (nHA) suspension. The study observed a significant increase in the remineralization effect when comparing the 5% and 10% concentrations. However, concentrations exceeding 10% did not yield substantial improvements in remineralization. This limitation can be attributed to the outer enamel layer composed of nHA, which acts as a barrier, impeding further diffusion into the carious lesion during the remineralization process within a short timeframe. Notably, the concentration of calcium in the nHA solution surpassed that of the micro hydroxyapatite solution. The study also revealed that the maximum mineralization effect occurred at a pH of 4.0, while a pH of 7.0 exhibited the least mineralization. In addition to its remineralizing properties, nHA was found to enhance the microhardness of teeth. Moreover, there is evidence suggesting a synergistic relationship between nHA and fluoride in the remineralization process. The use of a 0.05% sodium fluoride mouth rinse containing nano-hydroxyapatite demonstrated efficacy in remineralizing early carious lesions.

A comparative study conducted by Daas *et al* (2018) examined the efficacy of nano-hydroxyapatite (nHA) paste and fluoride varnish in the remineralization process. Surprisingly, the study revealed no significant difference between the two agents in terms of their remineralization effects. In the context of primary teeth, the remineralization effects of GC Tooth Mousse and nHA toothpaste were found to be comparable to that of 1000 ppm fluoride toothpaste. This evaluation was based on surface microhardness measurements conducted on artificial caries (Kasemkhun *et al.,* 2021). Furthermore, toothpaste containing nHA exhibited the highest rate of remineralization, followed by bioactive glass, CPP-ACP (*casein phosphopeptide-amorphous calcium phosphate*), and fluoride. Notably, significant disparities were observed in terms of surface microhardness and the formation of hydroxyapatite crystals after treatment in demineralized tooth samples across all experimental groups (Figure 4) (Geeta *et al*, 2020).

Elembaby et al., 2022 [95]	In-vitro	Effect of nHA incorporation into resin infiltrant on the mineral content, surface tomography, and resin tag penetration of demineralized enamel.	High-quality resin tags in demineralized enamel with enhanced mineral density, resin penetration and smooth surfaces
Wahba et al., 2022 [96]	In-vitro	Comparison of fluoride varnish, fluoride mouthwash, Self- Assembling Peptide (P_{11}-4), CPP-ACP, and nHA in prevention and arrest of Primary Tooth Enamel Lesions	Fluoride varnish, fluoride mouthwash showed caries-preventive effects whereas other agents did not show any effect
Sebastian et al., 2022 [97]	In-vitro	Comparison of CPP-ACP, nHA and Calcium Sucrose Phosphate (CSP) on artificial enamel lesion	CSP showed highest surface microhardness followed by nHA and CPP-ACP
Erdilek et al., 2022 [98]	In-vitro	Comparison of remineralization potential of fluoride gel, sodium fluoride toothpaste, and homemade nHA paste on artificial early enamel caries.	Homemade nHA paste had a enhanced remineralization potential of early enamel caries lesions
El-Gar et al., 2022 [99]	In-vitro	Assessment of biocompatibility and antibiofilm activity of suspension of nHA of large nanoparticle size (NHA-LPS) and nHA -small particle size (NHA-SPS)	NHA-LPS suspension showed enhanced bacterial adhesion and biofilm thickness in compared to NHA-SPS.
Atef et al., 2022 [100]	In-vitro	Effectiveness of diode laser, fluoride varnish and nHA on the enamel microhardness and microstructural alterations of the primary teeth enamel was assessed	All the remineralizing agents were equally effective in increasing microhardness and maintaining enamel microstructure integrity.
Eliwa et al., 2022 [101]	In-vitro	Comparison of remineralization potential of nano-seashell, nano-pearl, and nHA pastes with fluoride-based toothpaste.	Enamel surface microhardness was highest in fluoride-based toothpaste, nano pearl paste, nano-seashell paste and nHA.Fluorescence was decreased to greatest in nano-seashell, fluoride-based toothpaste, nHA pastes and least in nano pearls.

Figure 4. Summary of recent evidence on nHA in dental caries prevention (Geeta *et al*, 2020).

In a comparative investigation focusing on the remineralization rate of nano-hydroxyapatite (nHA), NovaMin, and amine fluoride in artificial enamel caries, all three agents demonstrated efficacy in remineralizing the artificial lesions. However, the utilization of nHA toothpaste yielded a more pronounced effect, significantly reducing the depth of the lesions when compared to amine fluoride toothpaste and NovaMin (Manchery *et al.,* 2019). Furthermore, an in-situ study examined the effectiveness of a dental lotion containing 5% nHA in remineralizing early caries and inhibiting enamel demineralization. The intervention involved the application of the lotion twice daily for a duration of 6 months in patients undergoing fixed orthodontic treatment. Results indicated that the dental lotion with nHA proved effective in promoting remineralization and curbing enamel demineralization within the specified timeframe (Amaechi BT *et al.,* 2021). Following the removal of fixed orthodontic treatment, the direct application of nano-hydroxyapatite (nHA) toothpaste and fluoride toothpaste over a period of 6 months consistently resulted in a reduction in the area of carious lesions, accompanied by an augmentation in enamel remineralization. (Badiee *et al.,* 2020).

In a clinical investigation, early caries lesions identified using the International Caries Detection and Assessment System (ICDAS) diagnostic criteria were subjected to treatment utilizing three distinct remineralization agents: Tricalcium phosphate paste (TCP), Fluoride varnish, and nano-hydroxyapatite (nHA). The efficacy of remineralization was assessed at the fifth week by recording the DIAGNOdent score of the initial caries lesion. The findings underscored the significant role played by all three remineralizing agents in facilitating the remineralization effect on occlusal and smooth surface caries. Notably, the application of nHA gel exhibited the most pronounced and statistically significant effect on early caries when compared to fluoride varnish and TCP paste (Alhamed *et al.,* 2020). However, it is important to note that the current body of clinical evidence remains insufficient to comprehensively evaluate the effectiveness of nHA in reducing dental caries formation and promoting remineralization of early caries lesions, especially when compared to in vitro studies. In addition to its remineralization properties, nHA may also demonstrate inhibitory effects on the colonization of Streptococcus mutans bacteria due to its excellent capacity for salivary protein absorption.

A research study unconvered that nano-hydroxyapatite (nHAp) has the potential to boost the formation of *streptococcus mutans* biofilm. Thi ocurrence is attributed to nHAP capacity to increase the transciption of glucosytransferase, leading to a higher production of insoluble glucans. However, variations inresults emerge when metal ions partially substitute calcium. In a different research, the influence of zinc ion incorporation, along with nHAP, *poly-acrylic acid*, and *grafted alendronate,* was evaluated. The result showed that zinc ions significantly enhanced the antibacterial effect against *S.mutans* compared to samples using nHAP alone. Additionally, another study indicated that toothpaste containing zinc-substituted nHAP exhibited a reduced antimicrobial impact against *S.mutans* when compared totoothpaste formulations incorporating substituions of strontium (Sr), magnesium (Mg), fluoride (F), and nHAP (Figure 5) (Imran *et al.,* 2023).

Table 3. Summary of the antimicrobial effect of hydroxyapatite nanoparticles.

Reference	Experimental Groups	Study Model and Design	nHAP Details	Main Findings on nHAP
Park et al. 2019 [45]	• 5% nHAP • 1% Sucrose • 5% nHAP with 1% sucrose • No treatment (negative control)	• Bacterial species *Streptococcus mutans*. • Model: *Streptococcus mutans* were incubated overnight in either brain heart infusion or basal medium mucin and exposed to the experimental groups. • Incubation: 24 h. • Assessment: cell survival by colony-forming unit and MTT staining.	• Particle size: (20–50 nm; 5–10% *w/v*). • Format: nHAP were commercially obtained (Alfa Aesar sol).	• nHAP enhanced biofilm formation in the presence of sucrose by increasing glucosyltransferase transcription.
Xu et al. 2020 [32]	• nHAP • Zn substituted nHAP • nHAP@Polyacrylic acid • Zn substituted nHAP@Polyacrylic acid • nHAP@ Alendronate-grafted polyacrylic acid • Zn substituted nHAP@Alendronate-grafted polyacrylic acid • Phosphate-buffered saline treatment (negative control)	• Bacterial species *Streptococcus mutans*. • Substrate: Bovine enamel specimens were cultured with *Streptococcus mutants*. • Model: Bacterial suspensions were cultured with 40 mg nanomaterials (experimental groups). • Incubation: 12 h at 37 °C. • Assessment: pH, cell viability by colony-forming unit and Fourier-transform infrared spectroscopy.	• Particle size: Zn substituted nHAP @ALN-PAA (Diameter: 3–10 nm, Length: 70–110 nm). • Format: Powder form of nHAP was obtained to synthesize Zn substituted nHA @ALN-PAA.	• Significant improvement in antibacterial activity against *Streptococcus mutans* was demonstrated in Zn substituted nHAP groups.

Figure 5: Summary of Antimicrobial Effects of Hydroxyapatite Nanoparticles (Imran *et al.,* 2023).

The safety of nanomaterials, including nano-hydroxyapatite (nHA), continues to be a subject of ongoing discussion and analysis, despite their widespread incorporation into various aspects of daily life facilitated by nanotechnology. The toxicity of nHA is influenced by a multitude of factors, such as the dosage and duration of exposure, aggregation and concentration, particle size and shape, surface area, crystal structure, and prior exposure history (Jeevanandam *et al.,* 2018). In a study conducted by Tay *et al* (2014), it was demonstrated that nHA particles can induce cytotoxic effects in oral epithelial cells. However, the permeability of nHA through the oral epithelium was investigated by Komiyama *et al.* (2019), who found that the presence of the stratum corneum can impede the entry of nanohydroxyapatite. These findings suggest that the infiltration of nano-hydroxyapatite into systemic regions through the oral epithelium is unlikely to occur. Thus, it is crucial to consider these cutting-edge studies when assessing the safety of nano-hydroxyapatite.

Conclusion

Nano-hydroxyapatite (nHA) is a versatile material renowned for its biocompatibility and structural similarity to inorganic bone components. Through an extensive review of the literature, it has been determined that nHAP exhibits the potential to enhance mineralization on early carious lesions by permeating the porous tooth structure resulting from the caries process, consequently elevating mineral content and hardness. The size of nHAP particles assumes a crucial role in the process of remineralization. Moreover, the incorporation of metal-substituted nHAP enables the attainment of antimicrobial effects. These distinctive properties position nHAP as a promising bioactive material for employment in the management of dental caries. Nevertheless, it is imperative to recognize that the utilization of nano-hydroxyapatite in dentistry is a relatively nascent field, warranting further investigation. Limitations persist, including the necessity for more conclusive evidence derived from clinical studies. Furthermore, the exploration of the underlying mechanisms of action and the synergistic potential of nHAP in combination with other agents represent areas requiring improvement. Future research endeavors should prioritize a comprehensive understanding of the precise mechanism by which nHAP facilitates remineralization and its potential synergistic interactions with agents such as fluoride. With its remarkable physical, chemical, and mechanical properties, nHAP emerges as an exceptionally promising material within the realm of modern dentistry.

Acknowledgements

All authors were glad to everyone who helped and encouraged the author in this work. The authors are grateful to all staff at Muhammadiyah University of Surakarta's Faculty of Dentistry

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