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Efficacy Of Nano Hydroxyapatite Derived From Marine Sources On Remineralization Of Enamel: A Systematic Review

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Background and Aim: Nano Hydroxyapatite (nHA) is a biocompatible material similar to natural bone and is used in dentistry to prevent and remineralize dental caries, etc. Marine sources like seashells, mollusk shells, crab shells, shrimp shells, fish bones, etc can create Nano-hydroxyapatite (nHA), a biomimetic material potentially useful for remineralization. This study aimed to systematically review the literature on the efficacy of marine-derived nHA in enamel remineralization compared to other remineralizing agents (e.g., fluoride, CPP-ACP, amorphous calcium phosphate)

Materials and Methods: This systematic review followed PRISMA guidelines, searching electronic databases from 1990 to July 2024 (Medline, Scopus, Cochrane). Only in vitro studies with Nano-hydroxyapatite from marine sources compared to other remineralizing agents were included. Two reviewers (VM, TGJ) screened articles, extracted data, and assessed study quality using the Quality Assessment Tool For In Vitro Studies (QUIN tool).

Results: Out of 43 identified articles, eight in vitro studies published between 2019 and 2023 were included, evaluating nHA from blood cockle shells, fish bone/scale, and oyster shells. Most of the included articles compared with commercially available CPP-ACP remineralizing agents. The QUIN tool revealed a medium risk of bias in seven studies and a low risk of bias in one.

Conclusion: While marine-derived nHA has shown promising results in remineralizing enamel lesions, the current evidence is limited by methodological shortcomings. Further well-designed research is needed to draw more definitive conclusions.

Keywords: Nanohydroxyapatite, seashell, oyster shell, fish scale, blood cockle shell, remineralization.

INTRODUCTION

Dental caries is a prevalent oral health condition characterized by the gradual breakdown of tooth enamel due to demineralization, leading to cavities and subsequent discomfort.[1] Nano hydroxyapatite (nHA) is a chemically stable ceramic compound with the formula $Ca_{10}(PO4)_6(OH)_2$. It possesses biocompatibility, better mechanical properties, and non-immunogenicity, etc.[2, 3] In dentistry, nHA has found applications in dentin hypersensitivity management, bleaching, implantology, the prevention and restoration of dental caries, augmentation of cell adhesion, etc.

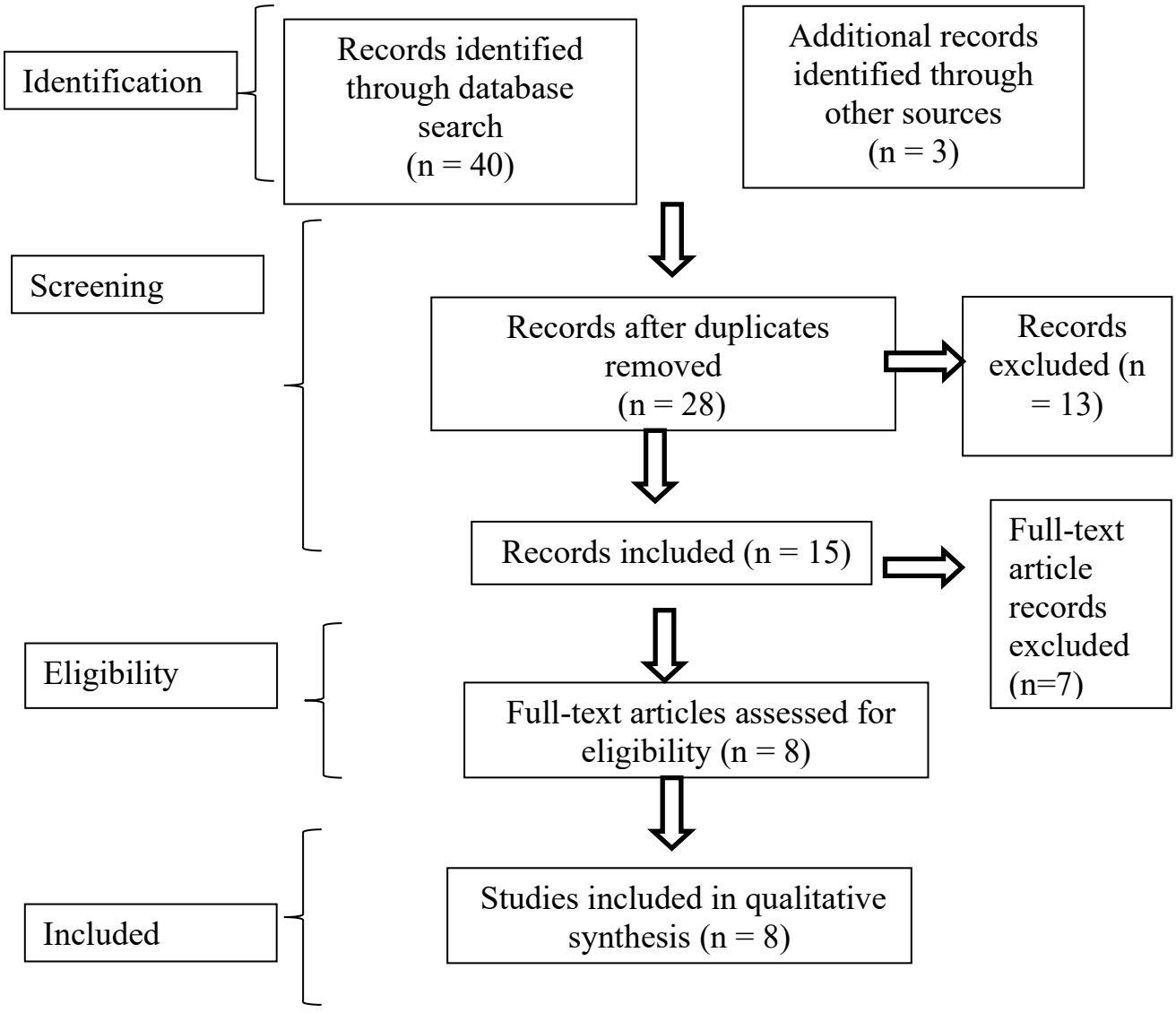
While traditional methods of obtaining nHA can be resource-intensive, biogenic waste shells offer a sustainable alternative such as poultry eggshells and shells from marine sources (sea shells, mollusk shells, crab shells, shrimp shells, blood clam shells, cockle shells, etc.). Millions of tons of these shells are discarded annually, contributing to environmental pollution.[4, 5] By converting these waste materials into biogenic materials such as hydroxyapatite, and chitosan, we can reduce waste and develop more sustainable dental solutions.

Though some studies in the literature researched the remineralization efficacy of marine-derived nHA on enamel, the evidence is limited. This study aimed to systematically review the literature on the efficacy of marine-derived nHA in enamel remineralization compared to other remineralizing agents (e.g., fluoride, CPP-ACP, amorphous calcium phosphate)

MATERIALS AND METHODS

This systematic review followed the PRISMA guidelines and was registered in the Open science framework (Figure 1).

Figure 1: **A flowchart of the literature search and the selection process according to the Preferred Reporting Items for Systematic Reviews**



Research Question

This study aimed to systematically review the published literature on the efficacy of Nano-hydroxyapatite derived from marine sources on enamel remineralization when compared to other remineralizing agents used (e.g., fluoride, CPP-ACP, amorphous calcium phosphate)

Eligibility criteria

The criteria for eligibility were based on using the following PICO framework: population (in

vitro and human studies), Intervention (nHA from marine sources), Comparison (Any other remineralizing agent), Outcome (enamel remineralization), open-access studies, and the studies were published in the English language.

Literature Search

A systematic electronic search of English language articles published between January 1990 and July 2024 was conducted, utilizing Medical Subject Heading terms in prominent databases including Medline (PubMed), Scopus, and Cochrane Central Register of Controlled Trials. Additionally, a comprehensive gray literature search was executed using OpenGrey. The following Boolean keywords were used: "Shells" AND "Nano Hydroxyapatite" AND "remineralization". Furthermore, a meticulous manual search of select journals such as the Journal of Endodontics, International Endodontic Journal, Biomaterials, Marine Drugs, Dental Materials, Clinical Oral Investigations, and others was performed. The selected articles were downloaded in Zotero (version 6.0.15). Subsequently, articles were meticulously screened by two independent reviewers (VM, SS). Any discrepancies regarding the inclusion or exclusion of retrieved articles were amicably resolved through deliberation with esteemed conservative dentistry and endodontic department senior faculty (PS, SJ). To address any missing data from the articles, diligent efforts were made to contact the authors via email. Only in vitro studies were deemed eligible for inclusion in this study, with review articles, animal experiments, and studies lacking a standardized reference test being excluded from consideration.

Data Extraction

Two authors (VM, PK) independently extracted data from the included studies using a customized data extraction form in Microsoft Excel. Subsequently, two additional authors scrutinized the extracted data (TD, SS). The extracted study characteristics encompassed author and year of publication, remineralizing agents derived from marine sources, any other remineralizing agent, number of teeth included, inclusion and exclusion criteria, blinding, interpreters, interobserver agreement, reference testing, and outcome data relating to sensitivity and specificity (Table 1).

Table 1 Characteristics of included studies

Author & year	Intervention & comparison group	Sample size	Evaluation	Statistical test	Conclusion
Eliwa M, Aidaros N, Kamh R.2022[14]	Nano HAp from cockle shell, nano-pearl paste, nano-hydroxyapatite (nHA) paste, and	80 premolar teeth	microhardness, remineralization of enamel by Diagnodent	Kruscall Wallis test, ANOVA test followed by post hoc test	Nano-seashell, nano-pearl, and nHA pastes have demonstrated comparable remineralization potential to fluoride-based toothpaste for

	fluoride				addressing initial non-cavitated enamel lesions in a noninvasive and productive manner.
Bhavan Ram U, Sujatha V, Vidhya S, Jayasree R, Mahalaxmi S. 2023[15]	Oyster-derived nHAp with or without proanthocyanin pre-treatment and CPP-ACP	197 maxillary or mandibular teeth	evaluated the dentinal tubule occlusion (TO), depth of penetration (DoP), and dentin permeability	Kruskal–Wallis test was used for inter-group comparisons. Intra-group comparisons were done using the Wilcoxon signed rank test.	Oyster-derived nHAp and proanthocyanidin pre-treatment groups showed significantly better tubule occlusion, increased depth of penetration, and reduced dentin permeability compared to CPP-ACP.
Hikmah N, Tanumihardja M, Nugroho JJ, Natsir N, Hamrun N, Kasim S.2023[15]	Nano HA from Blood clam shell powder, CPP-ACP	6 maxillary Premolar	Porosity of enamel, calcium, phosphate element, hydroxyapatite compounds after application of CPP-ACP and nHA After in-office bleaching.	Paired t-test, Mann-Whitman test	The study showed a decrease in the porosity, calcium, and phosphate and an increase in hydroxyapatite percentage after the sample was applied to the nHA synthesized from blood clam shells and CPP-ACP.
Hikmah N, Nugroho JJ, Natsir N, Rovani CA, Mooduto L. 2019[18]	Nano HA from Blood clam shell powder, CPP-ACP	12 enamel discs of upper premolars	evaluate the ca percentage before and after bleaching with 40% hydrogen peroxide	independent t-test	The nano-hydroxyapatite (nHA) derived from blood clam shells (anadara granosa) can increase the percentage of calcium after the

					application as an extra coronal bleaching material.
Hussien AO, Ibrahim SH, Essa ME, Hafez RM. 2023[17]	Nano fish bone powder, nano eggshell powder, control	34 maxillary third molar	evaluate the ca/p percentage before and after demineralization using EDX, SEM	paired t-test, independent t-test	Freshly prepared nano fish bone and eggshell solutions seem promising for remineralizing incipient enamel lesions.
Mathirat A, Dalavi PA, Prabhu A, GV YD, Anil S, Senthilkumar K. et al.2022[13]	Nano HA from fish bone (thermal), Nano HA from fish bone (alkali), CPP-ACPP (GC tooth mousse)	51 maxillary premolars	Characterization of HA and Evaluation of remineralization by diagnodent laser, dentin tubule occlusion using SEM, and microhardness by Vickers microhardness test	GraphPad Prism 8 and Origin Lab software	Thermally calcined HA and alkali-treated HA can induce mineralization and deposit minerals. Therefore, HA obtained from Epinephelus chlorostigma could be a potential biomaterial for treating early caries.
Mkhize SC, Onwubu SC, Mokhothu TH, Mdluli PS, Mishra AK.2023[16]	Nano HA from fish scale, Nano HA from eggshell, and Nano HA from milled fish scale	15 dentin model	Characterization of HA and Evaluation of dentin tubule occlusion using SEM	Oneway ANOVA	The HAp extracted from eggshells had higher crystallinity, superior buffering effects, and smaller particle size compared to the nHAp extracted from fish scales, making it a

					more favorable material for remineralization of teeth.

(n HAp- Nano hydroxyapatite, CPP-ACP- Casein phosphopeptide Amorphous calcium phosphate, HA- hydroxyapatite)

Quality Assessment of Selected Studies

The Quality Assessment Tool For In Vitro Studies (QUIN tool) was used to assess the quality of the selected studies. The tool includes 12 points, scoring, and grading options to allow clinicians to evaluate the quality of in vitro studies. It shows good content validity and reliability. Decisions about the selection of answers such as 2=Adequately specified 1=Inadequately specified 0= Not specified for each of the leading questions were determined after careful consideration by four experts. These are the following key criteria when assessing a study: explicitly defined aims and objectives; a comprehensive explanation of sample size calculation; a detailed account of the sampling technique employed; specification of the comparison group; a thorough explanation of the methodology; details of the operator involved; randomization procedure; method of outcome measurement; specification of the outcome assessor; utilization of blinding where applicable; the statistical analysis used; and the clear presentation of results. The studies were categorized as exhibiting high, medium, or low risk of bias based on their attained scores, with scores above 70% denoting low risk, scores between 50% and 70% indicating medium risk, and scores below 50% portraying high risk of bias. The final score was derived from the formula (total score × 100) / (2 × number of criteria applicable).

Data Analysis

The quality of the in vitro studies was appraised using the QUIN tool, comprising 12 items with scoring and rating options, facilitating investigators in evaluating the quality of in vitro studies. Four authors (VM, SS, PS, SJ) scrutinized the included studies and assessed them based on the criteria established by the tool. Among the included studies, only one exhibited a low risk of bias (RoB), whereas seven were categorized as having a medium risk of bias (Table 2).

Table 2 Data analysis

QUIN Questions	Mkhize et al.2023	Hikmah N, Nugroho JJ, Natsir N, Rovani CA, Mooduto L. Journal of Dentomaxillofacial Science. 2019	Hikmah N, Tanumihardja M, Nugroho JJ, Natsir N, Hamrun N, Kasim S. 2023	Mona Essam El Din Eliwa, et al. 2022	Mona EL Saied Essa, et al. 2023	Nugroho et al. 2019	Mathirat, A.; Dalavi, P.A.; Prabhu, A.; G.V., Y.D.; Anil, S.; Senthilkuma et al.2022	Bhavan Ram U, Sujatha V, Vidhya S, Jayasree R, Mahalaxmi S. 2023.
Clearly stated aims/objective	2	2	2	2	2	2	2	2
Detailed explanation of sample size estimation	0	0	1	0	1	0	0	0
Detailed explanation of the sampling technique	1	1	1	1	1	1	1	1
Detail of comparison group	2	2	2	2	2	2	2	2
A detailed explanation of the methodology	2	2	2	2	2	2	2	2
Operator details	0	0	0	0	0	0	0	0
Randomization	NA	NA	1	NA	NA	NA	NA	0
Method of measure	2	2	2	2	2	2	2	2

ment of outcome								
Outcome accessor details	NA	NA	NA	NA	NA	NA	NA	NA
Blinding	0	0	0	0	0	0	0	0
Statistical analysis	1	1	1	2	2	1	1	1
Presentation of Results	2	2	2	2	2	2	2	2
Total score	12	12	14	13	14	12	12	12
Items valid	10	10	11	10	10	10	11	11
ROB%	60	60	63.63636	65	70	60	54.54545	54.54545
ROB	Medium	Medium	Medium	Medium	Low risk	Medium	Medium	Medium

Results

Selection of studies

A total of 43 articles were found through electronic databases and manual searches. After removing duplicates, 28 records were included. Following the screening of abstracts, 15 full-text articles were reviewed. After reading the full texts of these 15 articles, only 8 were included in the final analysis. This was due to the use of Nano calcium, calcium, and hydroxyapatite in a few studies.[6-11] Additionally, one article[12] did not specify which material component was used in their study.

Study characteristics

The analysis encompassed eight in vitro studies published between 2019 and 2023. Four articles derived nanohydroxyapatite from blood cockle shells, three from fish bone and scale, and one from oyster shells. Most included studies compared the remineralization efficacy with a commercially available casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) remineralizing agent**.**

Quality Assessment of Included Articles Risk of Bias

A total of eight articles were examined in the context of this systematic review. Within this sample, one article was deemed to have a low risk of bias, while seven articles were associated with a moderate risk of bias. Notably, none of the included articles except one article employed randomization. It is worth noting that incorporating a randomization process in the assignment of interventions, or the selection of samples for analysis by the investigator or statistician, could have contributed to mitigating bias in these studies.

In most in vitro studies, extracted premolar teeth were used, except for three studies[13-15] which used molar teeth and dentin models. The thickness of each dentine slice ranged from 3 to 4 millimeters. Creating artificial lesions has advantages over natural lesions because they are reproducible. Artificial carious lesions were prepared using demineralizing solutions, phosphoric acid, or bleaching agents to induce enamel demineralization, followed by immersion in artificial saliva.[13-19] Remineralizing agents provided calcium and phosphate ions, with CPP-ACP being the most widely used, serving as a source of calcium and hydroxyl ions for enamel remineralization. The remineralization cycle ranged from 1 week to 4 weeks.

Discussion.

The treatment approach for dental caries involves a minimally invasive technique that comprises enamel remineralization. The reversal of early carious lesions is essential in preventing and repairing lesions.[18, 20] While fluoride therapy has traditionally been favored as an effective non-invasive treatment for early carious lesions, it has notable limitations. The restricted permeability of fluoride hinders the elimination of the opaque white appearance, thereby impacting esthetics. Furthermore, improper fluoride intake can lead to biotoxic effects, including the development of fluorosis.[21, 22] Remineralizing agents such as hydroxyapatite, casein phosphopeptides, amorphous calcium phosphate, and other antibacterial components are commonly utilized in the prevention of tooth decay and the restoration of dental structure.[6, 23] Nano-hydroxyapatite, akin to the hydroxyapatite crystals found in human teeth, elevates the degree of remineralization, particularly in acidic environments, by augmenting the supply of calcium and phosphorus ions to the demineralized area.[24, 25] Elevating the salivary levels of calcium and phosphate ion concentrations stands as a potential method to foster remineralization and impede demineralization in the teeth.[26-28]

The literature examined in our systematic review asserts that nano-hydroxyapatite (nHA) demonstrates hydrophilic properties and possesses a larger surface area, facilitating enhanced wettability. This characteristic allows nHA to form a robust, thin layer on the enamel surface, thereby affording strong adherence to the tooth structure. Moreover, the calcium content in nHA derived from shells, for example, blood clams (anadara granosa) is notably higher at 53% when compared with CPP-ACP, which contains a mere 18% calcium (GC Tooth-Mousse).[15, 17, 19] Calcium hydroxide has a high pH that can affect remineralization.

The assessment of enamel remineralization and microhardness encompasses a range of techniques. As such there is no standard technique to evaluate the enamel remineralization and hardness of the enamel. Predominantly, included articles in the current study have relied on scanning electron microscopy (SEM), and hardness testing to evaluate enamel remineralization and surface microhardness analysis. SEM, an electron microscope designed for surface analysis of solid objects, utilizes a focused beam of low-energy electrons to provide qualitative insights.[29, 30]

In addition to SEM-based methods, certain included studies have employed DIAGNOdent demineralization detection for quantitative analysis. This methodology operates on the principle that a Diode laser with a 655nm wavelength, when directed at the dental surface, is absorbed by intraoral bacteria metabolites, resulting in red fluorescence emission. The fluorescence, reflected by the dental surface, is then quantified on a scale of 0 to 99, with higher values indicating a larger decay area. Notably, laboratory investigations have shown that DIAGNOdent's sensitivity and specificity are comparable to other techniques.[30, 31]

Furthermore, some of the included studies have examined the percentage of calcium ions using SEM EDX analysis. When used with SEM, this microanalytical technique facilitates qualitative (element identification) and quantitative (concentration measurement) analysis. The EDX X-ray detector measures emitted X-rays about their energy, and subsequent evaluation using a computer-based program allows for qualitative and quantitative determination of the elements present in the specimen.[32, 33]

In summation, Only Eight studies met the inclusion criteria, which included in vitro studies that had not been analyzed for systematic review. QUIN tool used for assessing the risk of bias in this systematic review is an improved version that provides better evidence about sources of bias and variation in diagnostic accuracy research.[34] The QUIN tool has recommended randomization and blinding to reduce bias in the interpretation of the results. However, all articles investigated did not mention whether the investigators or statistician were evaluated with or without the knowledge of the intervention and control groups, which increased the risk of bias in the outcome.

The studies included in the analysis shared a common limitation in lacking information on the impact of the actual oral environment, including oral biofluids and various microorganisms. Another crucial consideration is the effect of toothbrushing under real-world conditions, encompassing its impact on mechanical and chemical wear. Additionally, the absence of the natural process of dental caries development in the caries model presents a significant limitation. Despite favorable results obtained from the experiments, it is important to note that they were conducted solely in a controlled environment. Furthermore, in vitro studies on dentine discs do not encompass the effects of pulp fluid. Addressing these limitations in future studies is essential.

Recommendations for Future Research

Nano hydroxyapatite can be sourced from various marine outlets. Future research endeavors may involve comparing Nano-hydroxyapatite derived from different marine sources with commercially available products. Further studies are required to identify the most efficient nanohydroxyapatite variant among them. Random sample allocation is recommended to minimize bias, and it is advisable to increase the sample size using standard methodological techniques.

Conclusion

While marine-derived nHA shows promise for enamel remineralization, the current evidence is limited by methodological shortcomings, particularly biases in statistical analysis, blinding, and explicit mention of sampling techniques, which may affect the reliability of the findings. Consequently, further rigorous and well-designed research is essential to draw more definitive conclusions.

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