

Evaluation of Remineralizing Effects of Human Enamel using Biomimetic Waste from Nanomaterial Solutions of Fishbone(Fb) and Eggshell(Es) Incorporated with Chitosan Nanoparticles in Single Rooted Tooth - An In Vitro Study

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ABSTRACT

Aim: The study aimed to evaluate the remineralizing effects of two freshly prepared organic biomimetic waste nanomaterial [fish bone (FB) and chicken eggshell (ES)] solutions on partially demineralized human enamel surface using energy dispersive X-ray spectroscopy for quantitative Ca/P assessment.

Methodology: A total of 50 freshly extracted human single rooted teeth were collected to be used. The samples were divided into five equal groups (n=10) respectively.

Group 1: Control group

Group 2: Remineralization solution obtained from fish bone biomimetic waste

Group 3: Remineralization solution obtained from chicken eggshell biomimetic waste

Group 4: Remineralising solution obtained from Fishbone biomimetic waste + chitosan nanoparticle

Group 5: Remineralising solution obtained from Eggshell biomimetic was +chitosan nanoparticle

Results: The results shows remineralisation effects of human enamel using biomimetic wastes were significant in Group 4 remineralising solution obtained from (FISHBONE+CHITOSAN NANOPARTICLES) and least in Group 3 remineralising solution obtained from(CHICKEN EGGSHELLS) respectively.

Keywords: Biomimetic, Human enamel, Demineralization, Nanoparticles, Eggshell, Remineralizing Solution, Fish Bone

INTRODUCTION

Cariology allows prevention of dental caries and controls it in its initial stages that primarily cause demineralization of hard tooth tissues. However caries is actually a multifactorial tooth disease, stopping and even reversing its pathogenesis as early as possible is an ultimate goal reduction of risk of tooth cavitation and subsequent operative clinical tooth intervention ^[1].

The concept of minimally invasive dentistry recently dictate essential need for effective modalities to clinically remineralize early human enamel demineralized lesions. Recently, non-fluoride remineralization strategies were broadly categorized into certain approaches that repair carious defects by enhancing fluoride anticariogenic efficacy and enamel biomimetic regenerative technologies ^[2].

Evidently, calcium phosphate hydroxyapatite (CaPHA) is an inorganic fundamental formula for formation of human teeth and bones. Added to mineral tissue concentration determines elastic moduli and strength of hard tissues. These provide rigidity and stiffness of teeth that are subjected to complex masticatory stresses ^[3].

Clinically, the first sign of enamel caries develop as a white spot lesion which is characterized by an opaque apparently intact covering over a partially demineralized subsurface. Based on current research, fluoride products were effective in enamel remineralization but they are inefficient in stimulating organised tooth apatite crystals. Consequently, biomimetic remineralization was an attempted therapy to shift from tooth reparative concept to regenerative hard tissue biomineralization, where the demineralized dental structures were replaced biologically ^[4].

Unlike bone or dentine, enamel regeneration seems challenging; as the mature acellular enamel never remodels a resorb itself or resorbs.

Recent advances in tissue engineering have yielded biomimetic materials that have demonstrated potential effect for regenerating the hierarchy of enamel microstructures.

Extracted CaPHA from sustainable natural primary sources seems a reliable, highly productive and feasible alternative for synthetic HA. Those CaP food waste reservoirs were mainly obtained from bones (of fishes and mammals) and biogenic (BioHA) resources like (eggshell, seashell and whelk) Out of all, Fish bones revealed rich content of calcium, phosphate, and carbonate; which advocates it as reliable natural sources for production of predictable biocompatible HA. Chicken eggshell extract represents a marvelous source for a cost effective HA due to munificent content of Ca oxides and carbonates ^[5].

Evidently, nano biomaterials reported more abundant Ca, fluoride (F) and P ion release profiles compared to that of micro-particles for remineralization of hard dental structures. Therefore, evaluation of remineralization potential of tentative elaborated FB and ES nano biomimetic solutions was a crucial feasible issue to be studied. Chitosan is considered as biodegradable material; those by-products are non-toxic. It acts as a functional group which induces polycationic mucoadhesive polymers that allows sustained release of bioactive substances over a while ^[7].

Moreover, the presence of a primary amine group at C-2 position allows modification of chitosan on repeat units. Chitosan was used for pretreatment of white spot lesions followed by the application of bioglass and the same with polyacrylic acid slurry. It was observed that chitosan improved the remineralisation ability, and the subsurface was seen to have higher mineral content ^[8].

EDX provides the atomic percentage (At%) or weight percentage (Wt%) of elements, with the Ca/P ratio serving as a primary indicator of hydroxyapatite, which typically drops during demineralization and increases upon remineralization.

Demineralization Detection: Acidic environments (below pH 5.5) break down enamel, reducing Ca and P concentrations, which EDX detects as a decrease in elemental weight percentages ^[27].

Remineralization Evaluation: EDX can quantify the mineral gain from treatments like fluoridated toothpaste, CPP-ACP, or bioactive glass, revealing increased Ca and P levels and the presence of fluoride ^[27].

Bander et al stated that EDX analyses were significant in assessing the quantitative analysis of calcium and phosphorus content after demineralisation and remineralisation effects ^[9].

MATERIALS AND METHODS

Study Design: An In vitro study was conducted in Adhiparasakthi dental college and hospital. A total of fifty freshly extracted human single rooted teeth were collected, and divided into five equal groups (n=10).

Group1: Teeth represents a control group, assessing normal quantitative content of calcium (Ca) and phosphorus (P).

Group2: Teeth were immersed in a freshly prepared demineralizing solution for 96 hours (i.e. four days), then investigated by EDX. The teeth were again soaked in a freshly elaborated remineralizing fish bone nanoparticle solution for 189 hours (i.e., seven days), then reevaluated by EDX.

Group3: Teeth were demineralized with the same regime as group 2, then remineralized in a freshly prepared chicken eggshell nanoparticle solution for seven days, then reassessed by EDX.

Group4: Teeth were immersed in a freshly prepared demineralizing solution for 96 hours (i.e. four days), followed by EDX investigation. Then the teeth were again soaked in a freshly elaborated remineralizing fish bone nanoparticle solution with chitosan nanoparticle for 189 hours (i.e., seven days), then reevaluated by EDX.

Group5: Teeth were immersed in a freshly prepared demineralizing solution for 96 hours (i.e. four days), then EDX investigation took place, the samples were again soaked in a freshly elaborated remineralizing chicken eggshell nanoparticle solution with chitosan nanoparticle for 189 hours (i.e., seven days), then reassessed by EDX.

Nano FB Powder Remineralizing Solution Preparation :

Nile Tilapia fish bones were bought from the local market, cleaned from organic debris by dipping it in boiling water and left to completely dry at room temperature. Then, FB were calcined for five hours in an electric furnace at 900 °C and left to cool for 15 minutes. The FB ashes were ground, sieved to fine powder (100-250 µm, brown color) and collected in a sterile container and that refined the particles to nanoscale (white yellowish color). Obtained FB nanoparticles were analyzed with EDX for percentage of elemental composition, while the particle size and shape of the powder was analyzed by SEM (X 400 magnification). One gram of the elaborated Nano calcined FB powder was dissolved into 33.3 mL of deionized sterile water and the clear fluid on the top was collected to be used as a freshly prepared 3% FB remineralizing solution ^[10].

Nano ES Powder Remineralizing Solution Preparation:

Chicken eggshells were washed with distilled water, soaked in 100 °C boiling water for 10 minutes to remove the membrane, dried at room temperature and homogeneously ground in a sterile mortar and pestle. Elaborated ES particles were analyzed by SEM for percentage of elemental composition and by SEM (X400 magnification) the particle size. A 3% ES remineralizing solution was prepared by blending one gram ES Nano calcined powder in 33.3 mL of deionized sterile water and on the top of the mix the clear solution was collected for use ^[10].

Teeth Preparation

The 50 selected teeth were cleaned from any hard or soft debris using an ultrasonic scaler, then careful enamel (E) examination was performed using a magnifying eyeglass to exclude any cracks,

white spot lesion, caries, hypoplasia, or other enamel defects. Throughout the study steps, those specimens were individually placed in ready-made artificial saliva and stored at 37°C in an incubator. In the Cervical third of buccal enamel surface of each tooth specimen a square window (3 × 3 mm) was drawn. Furthermore, the rest of the specimen was wrapped by a wax sheet after drying. Afterward, prepared specimens were stored in separate test tubes containing a freshly prepared artificial saliva [pH 6.57; prepared by blending of: Sterile deionized water 99.6 %, Potassium chloride 0.12%, Sodium chloride 0.08%, Magnesium chloride 0.01%, Carboxy-methyl cellulose 0.10%, Dibasic potassium phosphate 0.03% and Calcium chloride 0.01%] for 48 hours at 37°C in the incubator ^[11].

Demineralization Regimen

Group 2 and 3 teeth specimens were removed from artificial saliva, and they were individually immersed in test tubes containing a freshly prepared demineralizing (daily changed to prevent saturation, pH= 4.2; 2.2mMol calcium chloride, 0.05 M, acetic acid 2.2 mMol sodium phosphate and 1 M potassium hydroxide) solution at 37°C for 96 hours. Afterward, specimens were removed, rinsed for 5 seconds with distilled water to stop the effect of demineralizing solution and stored again in artificial saliva at 37 °C incubator until subsequent study step ^[12].

Remineralization Regimen

Group 2 and 3 demineralized specimens stored in artificial saliva were removed and they were again separately immersed into their respective freshly prepared E remineralizing solution [pH 7; 1.5 mMol calcium chloride, 0.15 mMol potassium chloride and 0.9mMol sodium phosphate,(group 2 for FB and group 3 for ES) for 12 min. in an incubator at 37 °C once a day. Afterward, those specimens were removed, rinsed for 5 sec. with distilled water and incubated for the rest of the day in artificial saliva at 37 °C. The remineralization regimen of specimens was daily repeated for 7 consecutive days using freshly prepared FB and ES solutions (for group 2 and 3 respectively ^[12].

Remineralization Solution Incorporated with Chitosan Nanoparticle Preparation

Chitosan solution was prepared by dissolving 0.1 -- 0.2% (w/v) chitosan in 1% (v/v) acetic acid under continuous stirring until completely dissolved. The pH of the solution was then adjusted to 4.6–5.5 using NaOH. For the preparation of the nanoparticle remineralization solution, ES nanoparticle solution was added dropwise into the chitosan solution under constant magnetic stirring, maintaining a mass ratio of ES to chitosan nanoparticles of 4:1. The mixture was stirred continuously for 30–60 minutes to ensure proper interaction and stabilization of the nanoparticles. The resulting suspension was centrifuged at 15,000 rpm for 30 minutes, and the supernatant was discarded. The pellet obtained was washed with distilled water and then redispersed to obtain the final nanoparticle remineralization solution. The nanoparticle size achieved in the remineralization solution ranged from 50 to 200 nm ^[13].

Specimen Edx Analysis

Following the study design and in each group, EDX quantitative evaluation of Ca and P content for teeth specimens was performed.

Statistical Analysis:

Table 1: Paired sample t-test of the difference b/w Ca and P level of remineralized teeth enamel after demineralization regime within groups.

Group	Group 1		Group2		Group 3		Group 4		Group 5	
	Control		FB		ES		FB+CS NP		ES+CS NP	
	Mean		Mean	SD	Mean	SD	Mean	SD	Mean	SD

Ca level	30.4	40.6	1.59	35.6	2.31	43.89	1.71	36.5	1.7
P level	27.7	18.3	0.91	18.23	0.17	21.4	1.1	19.3	0.21

Significant: $P < 0.05$

Table 2: Independent sample t-test of the remineralization efficacy of fishbone vs eggshell and nanoparticle incorporated with remineralizing efficacy of fishbone and eggshell

Groups	Ca level		P level	
	Mean	SD	Mean	SD
Fishbone vs Eggshell	6.201	0.91	4.31	0.71
Chitosan nanoparticle s incorporate d fishbone vs eggshell	7.31	1.12	5.29	0.97

Significant $p < 0.05$

RESULTS

The ph of used freshly prepared remineralizing solution was daily rechecked and their measured average values were

8.9 - FB

7.4 - ES

9.8 - FB+CSNP

8.6 - ES+CSNP

These shows remarkable alkaline nature of remineralizing solution. obtained EDX data for normal human sound enamel (group 1), initially demineralized enamel, nano FB powder, nanoES particles, FB remineralized enamel (group2) and ES remineralized enamel (group3) was pertained to Ca and P mineral content percentage.

Statistical EDX analysis showed that mean values \pm SD of normal enamel Ca was 30.4% and that of P was 27.7% (group1) and revealed obvious significant differences among all recorded mean values for all groups ($p < 0.05$). Paired sample t-test (table1) and independent sample t test (table 2) revealed significant difference between Ca and P level of remineralized teeth E after demineralization regime within groups (P -value < 0.05); as the FB+CSNP potential for Ca remineralization was significantly higher than that of FB.

MEAN % VALUE OF CA & P MINERAL CONTENT

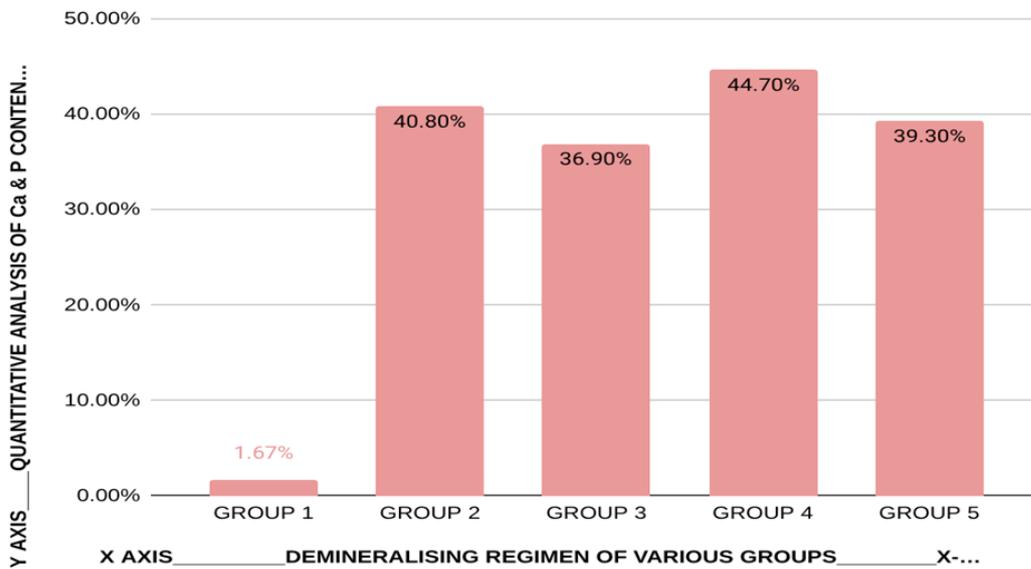


Figure 1: Shows mean% value of ca and p mineral content: demineralisation regimen

MEAN % VALUE OF Ca & P CONTENT REMINERALISATION REGIMEN

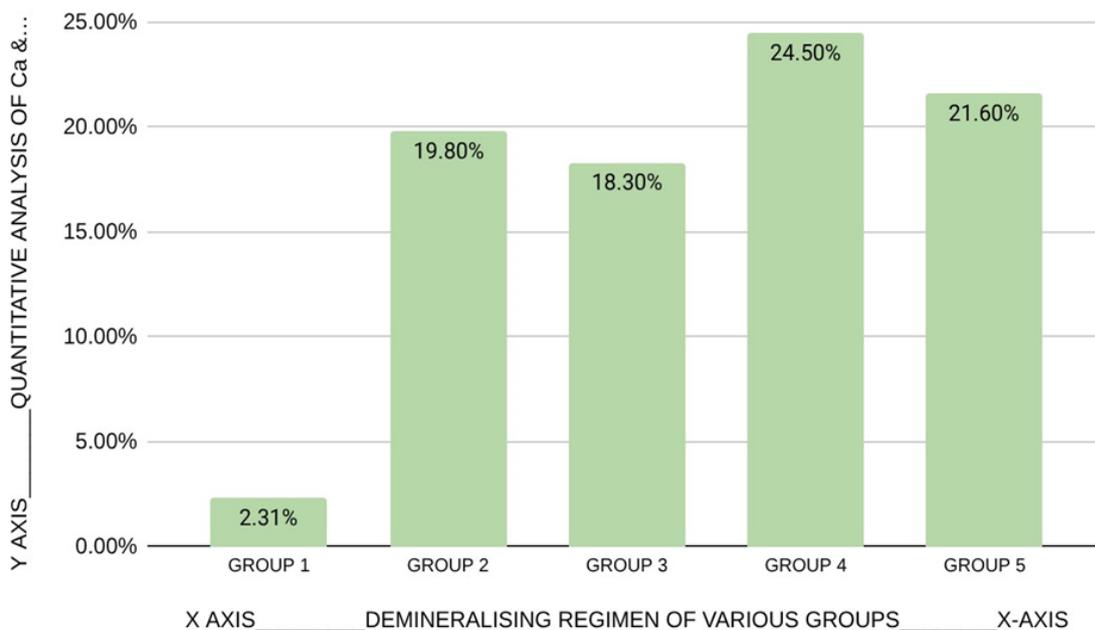


Figure 2: Shows mean % value of ca and p content : remineralisation regimen

DISCUSSION

Structurally, human hard E is a nanocomposite bioceramic characterized by significant specific resilience that protects the tooth hydroxyapatite (HA) from chemical, physical and mechanical oral environment damage.

The wonderful mechanical properties of enamel are associated with its hierarchical HA organization as well as its amazing thorough connection with the viscoelastic underlying dentin ^[10].

Accordingly, that E dynamic, still obscure mineralized system offers a wealth of information about basic principles for organic matrix-mediated biomineralization ^[11].

Those principles are potentially applied in engineering and biomaterial science fields for smart designing and development of biomimetic materials. Enamel is relatively stable in a healthy oral environment with the presence of saliva. the demineralization and remineralization of enamel are continuously taking place at the tooth-pellicle-plaque-saliva interface ^[12].

This concept provides the scope for remineralizing early enamel carious lesions. in recent decades, modern dentistry has increasingly emphasized non-invasive approaches for managing initial carious lesions, aiming to halve their progression and enhance the esthetics, strength and functionality of dental hard tissues ^[13].

These demineralisation and remineralisation can be partially reversed through a combination of ph neutralization and remineralization facilitated by incorporation of Ca/p components into hydroxyapatite crystals ^[14].

Advancements in nanotechnology have led to the incorporation of nanosized biomimetic apatite particles into dental products such as toothpaste and mouthrinses to enhance their therapeutic effects ^[15].

Nanohydroxyapatite (nHAP) stands out as an extensively researched biomaterial in the field of restorative dentistry, as evidenced by various studies. natural sources of calcium

carbonate, such as eggshells, have been harnessed for cost-effective synthesis of high-quality hydroxyapatite (HAP) ^[16].

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). by converting these waste materials into biogenic materials such as hydroxyapatite and chitosan, we can reduce waste and develop more sustainable dental solutions ^[17].

According to Salah M, Natural remineralizing agents like chitosan, which have a proven biocompatible nature, antibiotic properties, and biodegradability, have found a wide range of applications in regenerative dentistry for remineralization of teeth and regeneration of dental tissues like enamel and dentin ^[17]. Due to the active regions available in the structure of chitosan, it can be easily combined with various bioactive materials which act as a reservoir for ions required in remineralization ^[20].

According to Arifa MK, The ChNPs suspension was successfully synthesized and minimized human enamel demineralization after a cariogenic challenge, showing an interesting potential for use as an oral formulation for caries prevention ^[18].

According to Rincón-López JA, The ChNPs, they have the ability to act as bioadhesive scaffolds, aiding in the controlled release and facilitating the deposition of minerals for further repair of enamel microstructure ^[22].

According to leventouri TH, CHN nanocomposites effectively promoted remineralization of demineralized enamel through sustained release of calcium, phosphate, and fluoride ions ^[24].

Though obtained from previous literatures, it was evident that remineralisation was better with FB+CSNP, Thus the results from the current study indicates remineralisation effects of human enamel using biomimetic wastes were significant in Group 4 remineralising solution obtained from (FISHBONE+CHITOSAN NANOPARTICLES) and least in Group 3 remineralising solution obtained from(CHICKEN

Eggshells:

CONCLUSION

With limitations of this in-vitro study, the following may be concluded:

1. Demineralization regimes seem applicable for evaluation of remineralization potential of certain biomaterials.
2. Nanotechnology alters some biological, physical and mechanical biomaterial properties.
3. Biomimetic Ca/P of prepared nano-FB and nanoES remineralizing solutions seem promising, effective and low-cost remineralizing materials for enamel.
4. Biogenic HA resources deserve further studies to be clinically useful.
5. Chitosan nanoparticles provide a promising effect in penetration of enamel surface for mineral deposition, which directly influences remineralizing capacity of tooth structure.
6. Comparatively, evaluating the results of above study, remineralisation effects of human enamel using biomimetic wastes were significant in group 4 (remineralization solution of fishbone incorporated with chitosan nanoparticle) and least in group 3 (remineralisation solution obtained from biomimetic waste of chicken eggshell)

REFERENCE

1. Essa ME, El-Bolok AHM, Hammad HH. Biomimetic Human Enamel Remineralization Using Calcium Extract from Nano Fish Bone and Eggshell: An In Vitro Study. Egypt Dent J. 2023;69(2):1603-1611.
2. Jain N, Devadiga D, Rao RD, et al. Biomimetic Remineralization of Enamel and Dentin with Chicken Eggshell Slurry: An In Vitro Study. J Conserv Dent Endod. 2025;28(4):349-354.
3. Systematic review: In vitro remineralization effectiveness of eggshell extract on human teeth: systematic review and meta-analysis. PubMed. 2024.
4. Abd-elmonsif N, Gamal S. Potential remineralizing effect of cuttlefish bone and eggshell powders on demineralized human enamel. Pediatr Dent J. 2024;34(3):122-128.
5. Ruan Q, Moradian-Oldak J. Amelogenin and enamel biomimetics — review on biomimetic enamel reconstruction and protein-mediated mineralization. Journal of conservative dentistry and endodontics 2015.
6. Sauca Chalán NP, Paladínez Carrión YE, Palacios Astudillo IA. Biomimetic remineralization of enamel and dentin: literature review. International journal of biological macromol 2025.
7. Advanced review: Biomimetic mineralisation systems for in situ enamel restoration inspired by amelogenesis. Journal of conservative dentistry and endodontics 2021.
8. Zhao H, Zhang Q, Chu J. Effect of phosphate group on amelogenin peptide-mediated enamel remineralization. International journal of medical biochemistry 2024.

9. [Bandekar N, et al. The remineralization potential of fluoride, CPP-ACP, and chicken eggshell on enamel lesions: in vitro study..International journal of medical biochemistry 2025.](#)
10. [Enamel-like tissue regeneration using biomimetic enamel matrix proteins. International Journal of Biological Macromol. 2021.](#)
11. [Non-fluoride systems for enamel remineralization: biomimetic peptide scaffolds and calcium phosphate systems International journal of bioengineering 2025.](#)
12. [Biomimetic action of zinc-carbonate hydroxyapatite on enamel/dentin: review article on biomimetic hydroxyapatite technologies.International journal of biomedical application and sciences 2023.](#)
13. [Comparing remineralization potential of dentin powder vs. chicken eggshell powder on enamel lesions International journal of biomedical application and sciences 2024.](#)
14. [Advanced materials for enamel remineralization: overview of functional biomaterials and strategies. Frontiers Bioeng Biotech 2022.](#)
15. [Terzioglu P, Ögüt H, Kalemtaş A. Natural Calcium Phosphates from Fish Bones and Their Potential Biomedical Applications. Mater. Sci. Eng. C 2018; 91:899-911.](#)
16. [Mona El Saied Essa MES, Salem W, Hoda GH. Clinical Finding of Inert Collagen Sponge Saturated by Remineralizing Solution from Eggshell Powder Versus Ca-OH in Indirect Pulp Capping Measured by Cone Beam Computed Tomography Radiograph E.D.J. 2023;69\(1\): 3881:3894](#)
17. [Salah M, Kataia MM, Kataia EM, El Din EA, Essa ME. Evaluation of Egg Shell Powder as an Experimental Direct Pulp Capping Material. Futur. Dent. J.2018; 4:160-164.](#)
18. [Arifa MK, Ephraim R, Rajamani T. Recent Advances in Dental Hard Tissue Remineralization: A Review of Literature. International Journal of Clinical Pediatric Dentistry; 2019; 12 \(2\):139-144.](#)
19. [Zakrzewski W, Dobrzyński M, Zawadzka-Knefel A, Lubojański A, Dobrzyński W, Janecki M, Karolina Kurek K, Szymonowicz M, Wiglusz RJ, Rybak Z. Nanomaterials Application in Endodontics. Materials \(Basel\). 2021: 5296.](#)
20. [Best SM, Porter AE, Thian ES, Huang J. Bioceramics: Past, Present and for The Future. J. Eur. Ceram. Soc. 2008;28:1319-1327.](#)
21. [Abdallah R, Farouk H, Essa ME: Surface Topography and Thickness of Hybrid Layer Assessment after Dentin Pretreatment with Egg Shell Powder as Desensitizing Agent. E.D.J. 2018; 64\(4\): 1633-1641.](#)
22. [Rincón-López JA, Hermann-Muñoz JA, Giraldo-Betancur AL, De Vizcaya-Ruiz A, Alvarado-Orozco JM, MuñozSaldana J. Synthesis, Characterization and In Vitro Study of Synthetic and Bovine-Derived Hydroxyapatite Ceramics: A Comparison. Materials. Journal of conservative dentistry and endodontics 2018;11:333.](#)
23. [Eliaz N, Metoki, N. Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Applications. Materials.international journal of biomedical application and sciences 2017;10: 334.](#)

24. leventouri TH. Synthetic and Biological Hydroxyapatites: Crystal Structure Questions. Biomaterials 2006; 27: 3339-3342.
25. Haghgoo R, Mehran M, Ahmad M, Javad M. Remineralization Effect of Eggshell versus Nano-hydroxyapatite on Caries-like Lesions in Permanent Teeth (In Vitro) Journal of International Oral Health. 2016; 8 (4):435-439.
26. Mony B, Ebenezar AVR, Ghani MF, Narayanan A, Mohan AG. Effect of Chicken Egg Shell Powder Solution On Early Enamel Carious Lesions: An In vitro Preliminary Study. J Clin Diagn Res. 2015; 9(3): 30-32.
27. Tangboriboon N, Kunanurksapong R, Sirivat A. Preparation and Properties of Calcium Oxide from Eggshells via Calcination. Materials Science Poland. 2012; 30(4):313-322.
28. Gatti A, Camargo LB, Imperato JC, Mendes FM, Raggio DP. Combination Effect of Fluoride Dentifrices and Varnish on Deciduous Enamel Demineralization. Braz Oral Res. 2011; 25(5):433-438.