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Physicochemical Characteristics of Asian Moon Scallop Shell as a Tooth Remineralization Agent

Dewi M. Ramadhanti[1], Arlina Nurhapsari[2]*, Yayun S. Rochmah[3]

[1]*Master Program of Dental Sciences, Faculty of Dentistry, Sultan Agung Islamic University, Semarang, Indonesia .*
[2]*Department of Conservative Dentistry, Faculty of Dentistry, Sultan Agung Islamic University, Semarang, Indonesia .*
[3]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Sultan Agung Islamic University, Semarang, Indonesia.*

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ABSTRACT

Scallop shells have the potential to aid in the remineralization of the teeth. The aim of this study was to determine the physicochemical characteristics of hydroxyapatite (HA) extracted from Asian moon scallop shell and to analyse the enamel micropore closure after the application of HA gel. HA was synthesized from Asian moon scallop shell by the precipitation method, followed by 24-hour aging, and 8-hour calcination at 1000°C. the synthesized HA was characterized using scanning electron microscopy-energy-dispersive x-ray (SEM-EDX) spectroscopy, X-ray diffractometry (XRD), and Fourier transform infrared (FTIR) spectrophotometry. HA gel was formulated using sodium carboxymethyl cellulose (Na-CMC), and guar gum as gel base. The physical properties including the organoleptic properties, homogeneity, and pH of the HA gel formulation were tested. HA gel was applied to teeth and analysed using SEM-EDX. Results revealed that the HA synthesized from Asian moon scallop shell had a uniform particle size with an average size of 0.541 ± 0.026 µm, a calcium-to-phosphorus ratio of 1.63, and a crystallinity of 85.5%. Physical properties of the HA gel revealed a white, odourless, and homogeneous gel, with a pH above 5. SEM-EDX analysis of the teeth treated with HA gel indicated that the teeth treated with 20% HA gel exhibited the most compact micropore, with a micropore size of 0.912 ± 0.095 µm. The findings from this study suggest that Asian moon scallop shells may serve as an alternative material for tooth remineralization.

Keywords: Asian Moon Scallop Shell, Hydroxyapatite, Tooth Remineralization, Physicochemical Characteristics.

Introduction

The Remineralization of dental hard tissue refers to the process of supplying calcium and phosphate ions from external sources to the crystal cavities in demineralized tooth enamel, thereby enhancing the mineral content and increasing the hardness of the enamel.[1,2] Hydroxyapatite (HA) is a substance with the potential for tooth remineralization. HA acts as a remineralization agent by elevating the pH of the oral cavity to a neutral level and providing calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions in the demineralized area.[3,4] The demineralization process ceases once remineralization begins. The remineralization process reinstates lost HA minerals in the teeth. Remineralization occurs naturally if the pH of the salivary buffer is optimal.[4] HA ions supplementation can expedite tooth remineralization. Mineralized teeth exhibit enhanced strength, characterized by more compact micropore on the tooth surface.[5] HA is non-toxic and safe for ingestion. Its mineral constituents, specifically calcium and phosphate, or HA ions, are present in various natural materials in the environment.[5] Scallop shells are composed of 96.15% calcium oxide (CaO), making them an excellent source of calcium for the synthesis of bioceramics.

*Corresponding Author: E-mail: arlina@unissula.ac.id

Tel: +62 878-7880-0288

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Study on the hardness of tooth surfaces following the application of HA derived from scallop shell have been conducted. However, the optimal amount of HA that affects the diffusion rate of the material to the tooth surface has not been achieved.[6,7] Additionally, the characterization of HA materials is crucial to ensure their efficacy as remineralization agents.[8,9]

The current study aims to analyse the physicochemical properties of HA synthesized from Asian moon scallop shell, focusing on morphology, crystal structure, and diffusion to the tooth surface, using scanning electron microscopy (SEM).

Materials and Methods

Ethical approval

The study was approved by the ethical committee of the Komisi Etik Penelitian Kesehatan, Fakultas Kedokteran Gigi Universitas Islam Sultan Agung with reference number: 582/B.1-KEPK/SA-FKG/VII/2024.

Synthesis of hydroxyapatite

Asian moon scallop shells were used as a source of $CaCO_3$, they were obtained from refuse at the Semarang fish auction market. The precursor solution consisted of 25% ammonium hydroxide (NH_4OH) and 99.5% diammonium hydrogen phosphate ($[NH_4]_2HPO_4$) (Merck). HA and HA gel from Asian moon scallop shell were prepared at the Laboratory of Material Physics, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta.

Ten grams (10 g) of CaO powder were dissolved in 70 mL of distilled water at 60°C, the solution was stirred at 500 rpm for 1 hour. Similarly, 0.0648 mol of $(NH_4)_2HPO_4$ was prepared as a phosphate source by dissolving 8.5536 g in 70 mL of distilled water at 60°C, the solution was stirred at 500 rpm for 1 hour. The $(NH_4)_2HPO_4$ solution was titrated

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into the CaO solution at a rate of 1 mL/min. The titration was conducted while stirring on heated magnetic stirrer for 1 h.
The pH of the homogeneous solution of CaO and $(NH_4)_2HPO_4$ was controlled by the addition of 15 mL of ammonia solution (NH_4OH), the solution was distilled and maintained in alkaline condition (pH >9). The solution was made homogeneous by stirring at 500 rpm and at 60°C for 1 h. The solution was subsequently allowed to stand for 24 h, after which it was filtered over 24 h period. The filtrate was heated in an oven at 100°C for 8 h. The solid mass obtained was calcinated in a furnace at 1000°C for 8 h. The calcinated solid was thereafter referred to as hydroxyapatite (HA).

Physicochemical characterization of hydroxyapatite
The morphology of HA crystals was determined using Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX) (Joel JSM-6510LA-1400, Japan). The dimension of HA grain was determined using ImageJ software. Additionally, EDX enabled the determination of the Calcium-Phosphorus (Ca:P) ratio in the HA sample. The crystallinity of HA was determined using an X-ray diffractometer (XRD) (Bruker D8 Advance Eco, Germany). The XRD data were processed using Origin Pro 9 software. The functional groups, bond types, and chemical composition of scallop shell HA was determined by Fourier transform infrared (FTIR) spectrophotometry (Thermo Nicolet IS 10, USA) scanning over a wavenumber range of 4000-400 cm^{-1}.

Formulation of hydroxyapatite gel
A 6% concentration of sodium carboxymethyl cellulose (Na-CMC) was prepared in distilled water. The mixture was stirred at 500 rpm for 1 h until it became homogeneous. A suspension of 4% Guar gum was prepared in distilled water, and stirred at 350 rpm for 1 h until a homogeneous mixture was achieved. Glycerine was mixed with distilled water in a 1:1 ratio and stirred at 500 rpm for 1 h. The three preparations were thoroughly mixed and stirred for 1 h to produce a uniform gel base.
HA was dissolved in distilled water to make 10% and 20% solutions. The solutions were stirred at 500 rpm for 3 h. HA gel was formulated by incorporating HA solution into the gel base, followed by stirring at 500 rpm for 1 h.

Assessment of physical properties of HA gel
The physical properties of the HA gel formulation tested include; organoleptic properties, homogeneity, and pH. Tests for organoleptic properties and homogeneity were conducted on HA gels at concentrations of 10% and 20%. Organoleptic properties evaluated included consistency, colour, odour, and uniformity.

Sealing of dentine micropores with hydroxyapatite gel
Premolar teeth samples measuring 1 × 1 × 1 cm were prepared and embedded in acrylic media. The demineralization procedure involved the application of 37% phosphoric acid for 15 seconds to induce microporosity in the dentin. The remineralization procedure included the application of a base gel for the control group, alongside treatment groups using 10% and 20% HA gel. The application was done for 10 min, twice daily for 14 days. Subsequently, the teeth samples were subjected to SEM-EDX analysis to evaluate the closure of dentine micropores and the associated chemical structure of the teeth.[5]

Statistical analyses
Statistical analyses were performed using SPSS software (IBM SPSS, Armonk, New York, USA).

Results and Discussion

Natural HA shows promise for enhancing the strength of bones and teeth.[7,10] The synthesis of HA from natural materials involves a sequence of steps to produce optimized HA for application as a tooth remineralization agent. The synthesis procedure involves transitioning from the disc milling process to calcination at a temperature of 1000°C for 8 hours, followed by characterization of the resulting HA.[11,12]

Morphology of hydroxyapatite crystals
The SEM-EDX analysis of HA synthesized from scallop shell revealed a consistent HA morphology (Figure 1). Measurements of 52 particles from the SEM image taken at a magnification of 10000 using ImageJ, revealed that the average size of HA particles was 0.541 ± 0.026 µm (Figure 2a). EDX analysis revealed Ca:P ratio of 1.63 (Figure 2b). EDX mapping indicated that the distribution of Ca (red), P (green), and O (blue) was uniformly spread across the HA sample derived from scallop shell (Figure 2c). Consistent with earlier studies,[5,6] calcium predominated the HA group of scallop shell, followed by phosphorus and oxygen. This consistent morphology, with a microstrain size of 0.016 ± 0.002 µm, enhances the diffusion of HA into the micropores of the teeth.[13,14] The Ca:P ratio of 1.63 aligns closely with Ca:P ratio in natural bone, which ranges from approximately 1.6 to 1.73. An increased calcium-to-phosphorus ratio enhances the potential availability of calcium within the HA structure. Higher calcium level improve the likelihood of HA diffusion, thereby promoting tooth remineralization.
According to the findings of Syafaat and Yusuf (2019),[15] this Ca:P ratio increases the probability that the application of HA to the teeth will elevate their hardness to levels comparable to that of natural bone. The uniform distribution of calcium, phosphorus, and oxygen within HA indicated that the synthesized HA is consistent with natural HA of identical elemental composition. The calcium content of 59.63% and phosphorus content of 18.94% demonstrate the potential of HA as a promising alternative for tooth remineralization.[5]

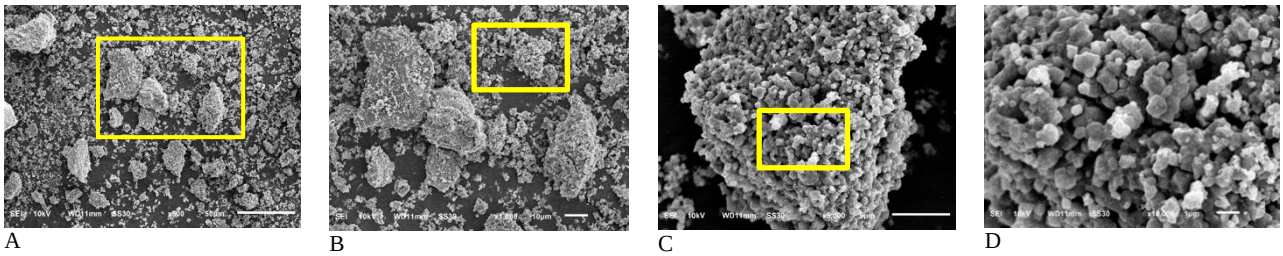
A B C D

Figure 1: SEM images of hydroxyapatite derived from Asian moon scallop shells. Magnifications (A) 500x, (B) 1000x, (C) 5000x, (D) 10000x

Crystallinity of hydroxyapatite from Asian moon scallop shell
The crystal structure of the synthesized HA was analysed using x-ray diffraction (XRD) technique. XRD analysis using Origin Pro 9 software for data processing, revealed strong diffraction peaks and high crystallinity of HA synthesized from scallop shell. The diffraction pattern obtained from the XRD analysis (Figure 3a) was compared to the crystal phase database standard (Joint Committee on Powder Diffraction Standards [JCPDS]). The results showed that the XRD diffraction pattern of the sample aligned with the HA peaks outlined in JCPDS data No. 09-0432. The synthesized HA exhibited diffraction peaks at 31.76°, 32.19°, and 32.89°, corresponding to the diffraction planes (211), (300), and (202). The measured HA particles exhibited a crystallite size of 43 ± 3 nm, with a lattice parameter a of 0.954 nm and a lattice parameter c of 0.698 nm (Table 1). The XRD results indicated

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a crystallinity level of 85.5%. These lattice parameters aligned with XRD pattern of reference HA, which exhibited a hexagonal crystal structure, specifically 9.43 Å for the lattice parameter a and 6.87 Å for lattice parameter c.[16] These findings demonstrated a high level of crystallinity of the synthesized HA, confirming that the produced minerals are exclusively HA, without the presence of other elements, as evidenced in the study by Cahyati *et al* (2024).[12]

Table 1: Crystal characteristics of Asian moon scallop shell hydroxyapatite

Parameter	Grid Parameters		C/P Ratio	Crystallite Size	Microstrain
	a	c			
Value	9.43 Å	6.87 Å	1.63	43 ± 3	0.016 ± 0.002

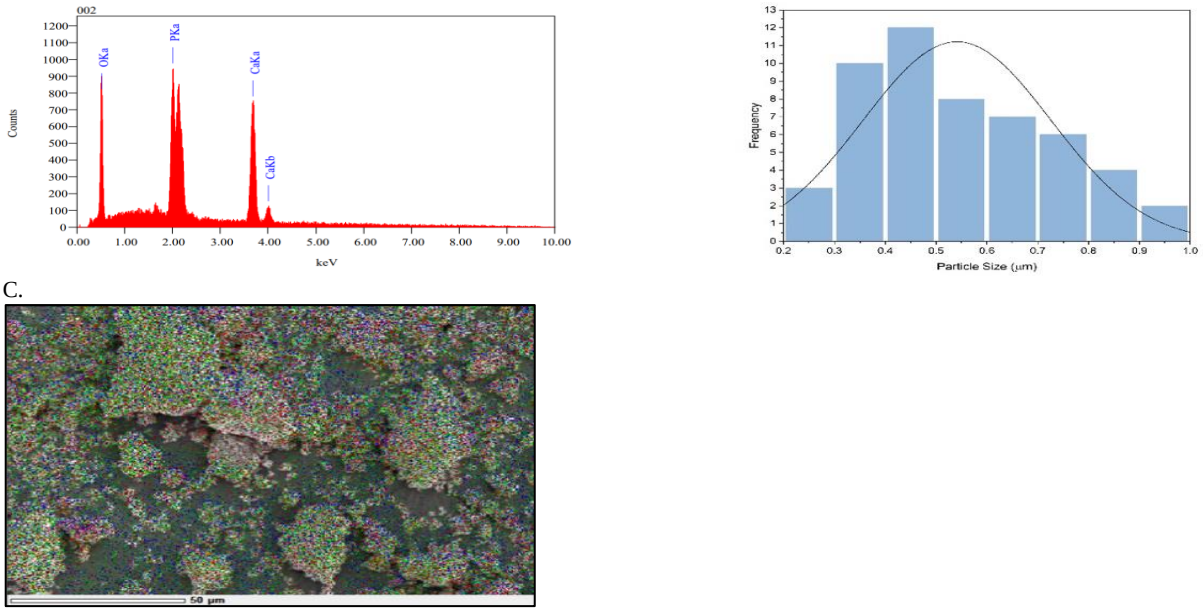

Figure 2: SEM-EDX analysis of hydroxyapatite derived from Asian moon scallop shells. (A) Hydroxyapatite Elemental Composition, (B) Hydroxyapatite Particle Size, (C) EDX image showing the uniform distribution of Ca (red), P (green), and O (blue) throughout HA

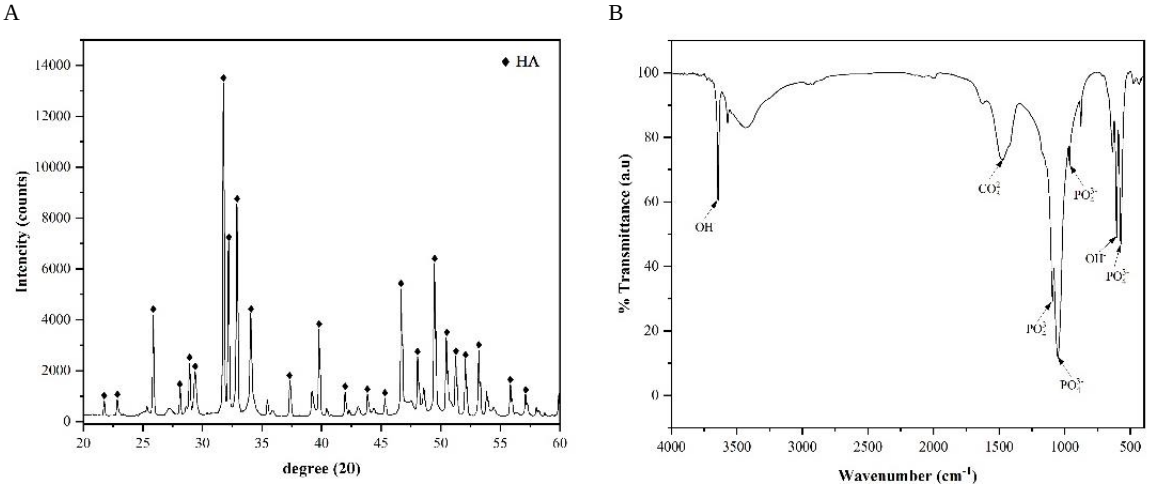

Figure 3: Crystallinity and chemical composition of hydroxyapatite from Asian moon scallop shell. (A) XRD Diffraction Pattern, (B) FTIR Spectrum

Chemical composition of hydroxyapatite from Asian moon scallop shell

The chemical composition of the synthesized HA was analysed using FTIR. The FTIR spectral data was compared with the spectra database (HR Nicolet Sampler Library). The FTIR spectrum of synthesized HA indicated that calcium and phosphate ions have been incorporated into the HA lattice structure (Figure 3b). The spectrum revealed a distinct absorption band at 1092.82 cm^{-1}, corresponding to the stretching vibration of the PO_4^{3-} functional group. Additionally, the absorption bands observed at 1050.08, 964.56, and 569.24 cm^{-1} were indicative of the bending vibrations of the PO_4^{3-} functional group. The absorption bands at 3646.57 cm^{-1} and 601.3 cm^{-1} correspond to the vibrations of the OH^- group, while the band at 1477.49 cm^{-1} indicates the vibrations of the CO_3^{2-} functional group. Therefore, the FTIR results confirmed the presence of the functional groups PO_4^{3-}, CO_3^{2-}, and OH^-, which are characteristic of HA material. The FTIR results corroborated the findings from the XRD, thereby confirming that the HA sample obtained is pure.[17]

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Quality of hydroxyapatite gel formulation

The formulation of scallop shell HA gel followed the synthesis of HA. The quality of the gel was evaluated through organoleptic assessment, homogeneity analysis, and pH measurement. The organoleptic assessment indicated that the HA gel presented a yellowish-white hue, it was odourless, and exhibited a semi-solid texture (Figure 4). The organoleptic properties demonstrated the suitability of HA gel for dental applications. The homogeneity test conducted by examining the distribution of the gel, showed that the 10% and 20% concentrations of scallop shell HA gel were homogeneous. The pH test on HA gel at 10% and 20% concentrations revealed a pH value of 12. A gel pH of 5 or higher signifies a uniform phase without clumping or bubbles. These qualities aligned with the SNI 12-3524-1995 standard, indicating a soft, homogeneous, odourless gel, free from air bubbles or any visible foreign matter, and a safe pH level for use in the oral cavity. this type of gel has been reported to be readily absorbed by teeth.[18]

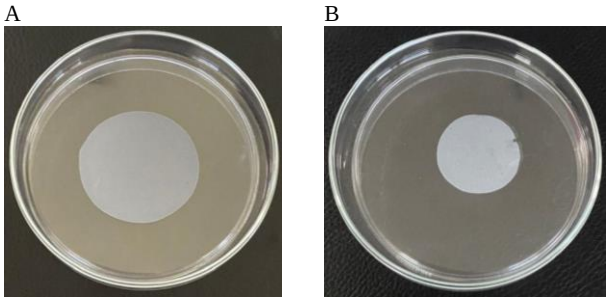
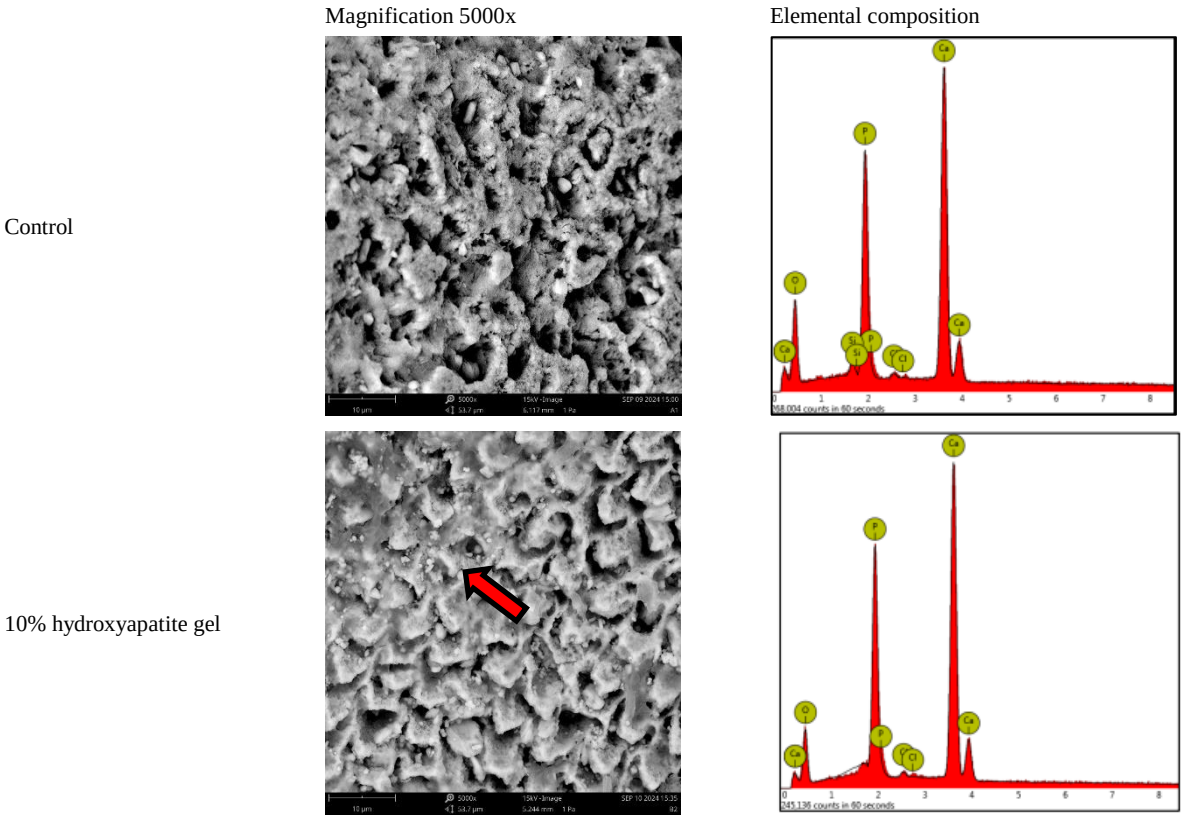


Figure 4: Hydroxyapatite Gel from Asian moon Scallop Shell. (A) 10% Concentration, (B) 20% Concentration

Effect of scallop shell hydroxyapatite gel on micropore closure

Teeth treated with scallop shell HA gel were subjected to SEM-EDX analysis, and SEM images were produced using ImageJ software. The resulting effect on micropore closure indicated that in the control group, where the base gel (placebo) was applied, the dentine tubules remained open following demineralization, with a micropore size of 3.003 ± 0.274 µm. In the group treated with 10% scallop shell HA gel, the teeth exhibited slight closure of the dentine tubules, with visible HA elements (indicated by the red arrow), showing a micropore size of 2.316 ± 0.264 µm. The group treated with 20% scallop shell HA gel demonstrated notable closure of dentine tubules, with observable HA elements (red arrow), and a more pronounced micropore closure, with a micropore size of 0.912 ± 0.095 µm (Table 2). These findings indicate that the incorporation of 20% HA gel resulted in a more effective sealing of the tooth surface micropores. The tooth remineralization process involves the diffusion of HA, enhancing tooth hardness. EDX analysis revealed that the primary elements present in the sample were calcium and phosphorus. Data presented in Table 2 indicate that Ca and P levels increased with an increase in HA gel concentration, meaning that the 20% HA group had the highest Ca and P contents of 59.63% and 18.94%, respectively, compared to the other groups. SEM images of the 10% and 20% scallop shell HA gel groups revealed visible Ca and P mineral deposits, highlighted by the red arrows in the image (Figure 5). The EDX graphs obtained were consistent with micropore closure, suggesting that a higher calcium and phosphate composition in the gel enhances the density of micropore closure following demineralization. Micropores generated during demineralization result in mineral dissolution in tooth enamel, forming nanogaps within the interprismatic space.[19] The diffusion of calcium and phosphate is influenced by factors such as contact time, crystallinity, the Ca:P ratio, HA microstrain size, and the method of application to the teeth.[20] The use of scallop shell HA gel, rich in calcium and phosphate has been shown to facilitate the diffusion of HA into the nanogaps of the interprismatic space, reducing micropore size as calcium and phosphate diffusion within the HA gel increases.[21] This indicates that Asian moon scallop shell HA gel may serve as an effective agent for tooth remineralization.



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20% hydroxyapatite gel

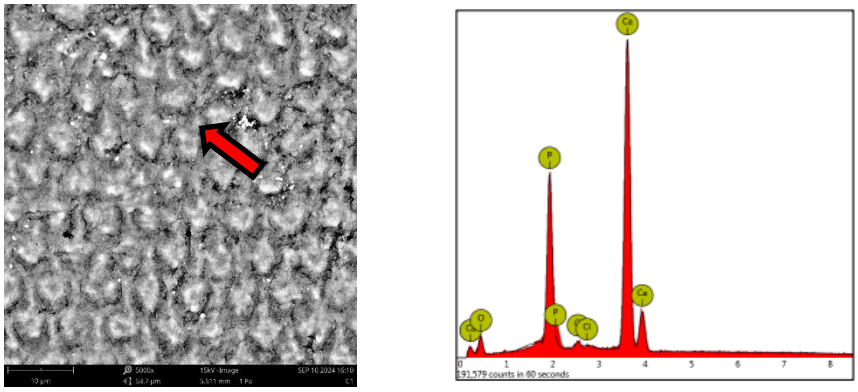

Figure 5: SEM images of tooth morphology and elemental composition post-application of Asian moon scallop shell hydroxyapatite gel at 5000x magnification

Table 2: Micropore size, Calcium and Phosphorus content of teeth treated with Asian moon scallop shell hydroxyapatite gel

Group	Micropore Size (µm)	Ca (%)	P (%)
Control	3.003 ± 0.274	38.60	16.45
10% hydroxyapatite gel	2.316 ± 0.264	44.89	18.35
20% hydroxyapatite gel	0.912 ± 0.095	59.63	18.94

Conclusion

The findings from this study have demonstrated that HA derived from Asian moon scallop shell may serve as a viable alternative agent for tooth remineralization. Characterization of the synthesized HA has shown that calcium is the predominant element in HA. SEM-EDX analysis of teeth treated with Asian moon scallop shell HA gel revealed that treatment with 20% gel resulted in a more effective micropore closure compared to the other groups. However, *in vivo* studies are recommended to assess the biocompatibility of HA gel on the oral mucosa surface.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original, and that any liability for claims relating to the content of this article will be borne by them.

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