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Research Article

Saltwater Fish Nanoparticles: Biological Effects on COL1A1 Expression in Fetal Mice and Tablet Formula Optimization

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Abstract

Saltwater fish nanoparticle-based tablets represent a novel nutritional strategy aimed at enhancing dental hard tissue density, particularly enamel. These tablets incorporate bioactive proteins, omega-3 fatty acids, and essential minerals such as calcium, phosphate, and magnesium to support enamel biomineralization. The nanoparticle formulation facilitates efficient cellular absorption, thereby increasing the expression of key enamel proteins like collagen type I alpha 1 chain (COL1A1) during enamel matrix development. This study investigated the effect of saltwater fish nanoparticles on COL1A1 expression in ameloblast cells and evaluated the physicochemical properties of tablets with talc concentrations of 1%, 5%, and 10%. Using a true experimental design with a post-test only control group, two groups of mice were assigned: a control group fed a standard diet, and a treatment group fed a standard diet supplemented with saltwater fish nanoparticles (2.17 mg/0.5 mL). Tablet formulations were analyzed across the three talc concentrations. Data were subjected to independent T-tests for COL1A1 expression and One-way ANOVA for physicochemical properties. Results revealed a significant reduction in COL1A1 expression in the treatment group. Additionally, talc concentration significantly influenced tablet physicochemical characteristics, with the 10% talc formulation exhibiting the most optimal properties. These findings suggest potential for nanoparticle-based nutritional interventions to promote enamel formation.

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1. Introduction

Dental caries is among the most prevalent diseases in children, arising from the demineralization of enamel (Pitts *et al*., 2017). One contributing factor is developmental defects of enamel (DDE) during the intrauterine period. DDE may result from nutritional deficiencies, such as calcium and omega-3 fatty acids, during pregnancy, which disrupt amelogenesis by impairing hydroxyapatite crystal formation and suppressing the expression of enamel matrix genes (Champiloma *et al*., 2025; Piekoszewska-Ziętek *et al*., 2025). Consequently, this leads to weakened enamel structure and an increased risk of childhood caries (Collignon *et al*., 2022). To address maternal calcium and omega-3 deficiencies, supplementation strategies have been developed. However, although tablets and capsules are available, their effectiveness remains limited due to low bioavailability and poor intestinal absorption (Padmanaban *et al*., 2025). Therefore, improved delivery formulations are needed to achieve optimal outcomes.

Nanoparticles, defined as particles measuring 1–100 nm, are increasingly explored to enhance bioavailability, modify drug delivery systems, increase stability of active compounds, and improve absorption (Mall *et al*., 2024). Recent studies suggest that saltwater fish nanoparticles may enhance enamel hardness; however, evidence remains insufficient and warrants further investigation (Putri *et al*., 2021). This highlights the need to better understand the impact of saltwater fish nanoparticles on enamel and to develop effective formulations for their delivery.

Saltwater fish are an abundant source of macro- and micronutrients essential for tooth development. Studies have shown that exposure to saltwater fish-derived nanoparticles during pregnancy can enhance osteoblast proliferation in the fetal alveolar bone of mice (Christiono *et al*., 2024b). Maternal supplementation with nano-calcium and vitamin D has also been reported to accelerate tooth development and eruption, likely by promoting enamel and dentin formation during intrauterine life (Sitosari *et al*., 2020). Additionally, research indicates that consumption of saltwater-based animal products can stimulate the formation of hydroxyapatite crystals resembling enamel (Xing *et al*., 2022). Saltwater Fish powder has been shown to accelerate odontogenesis in mouse fetuses by improving mineralisation and calcium transport into the enamel matrix, involving biomolecules such as Fatty Acid Binding Proteins (FABPs), amelogenin, and Alkaline Phosphatase (ALP) (Christiono *et al*., 2022; Christiono *et al*., 2023a; Christiono *et al*., 2023b; Christiono *et al*., 2023c). Furthermore, other studies demonstrate that saltwater fish nanoparticles increase enamel density and hardness during the intrauterine phase in mice (Putri *et al*., 2021; Christiono *et al*., 2021). These nanoparticles exhibited no toxicity in zebrafish embryos at concentrations ranging from 125 to 4000 µg/mL, indicating potential safety as a supplement for amelogenesis (Christiono *et al*., 2024a). Other nanoparticle forms like nano-hydroyapatite (nHAp) have also been shown to act as a biomimetic material that provides localized sources of Ca^{2+} and PO_4^{3-} ions, supporting the mineralization of the matrix produced by ameloblast and odontoblast (Pushpalatha *et al*., 2023). Notably, proline derived from saltwater fish has been shown to significantly upregulate COL1A1 expression through the Transforming Growth Factor beta/Suppressor of Mothers Against Decapentaplegic (TGF-β/Smad) pathway in the swim bladder and liver (Rong *et al*., 2022).

However, the effect of saltwater fish nanoparticles on Collagen type I alpha 1 chain (COL1A1) gene expression remains unclear, despite its critical role in developing dental tissues. Despite the promising effects of saltwater fish nanoparticles on enamel quality, their influence on COL1A1, a key gene involved in type I collagen synthesis during amelogenesis, remains poorly understood. Additionally, developing these nanoparticles into optimised oral dosage forms, beginning with tablets as a pilot project, is essential.

This study aims to provide novel insights into the biomolecular and pharmaceutical aspects of saltwater fish nanoparticles, contributing valuable information about their potential benefits in amelogenesis and formulation development.

Specifically, this research investigates the effect of saltwater fish nanoparticles on COL1A1 expression in fetal mice ameloblast cells. It evaluates the impact of talc on the physicochemical properties of saltwater fish nanoparticle tablets.

2. Materials and Methods

2.1 Materials

2.1.1 The equipment

The equipment used for evaluating COL1A1 expression included a light microscope (Olympus CX-21, New York) at 400× magnification, tweezers, glass slides, cover slips, a microtome, and a water bath. For the physicochemical assessment of the formulations, the following instruments were employed: an analytical balance, mortar and pestle, metal tray, oil

paper, tray dryer (KT500), sieve no. 12, granule flow tester (GTB 0.2 A, Erweka®, Heusenstamm, Germany), tapped density tester (SVM-22 0.5 A, Erweka®, Heusenstamm, Germany), measuring cylinder, single punch tablet machine (Delta Model VFD007S21A), friability tester (B-One CS-1), hardness tester (B-One BTH-3), and a disintegration tester (Erweka® Type ZT 302, Germany).

2.1.2 The materials

Materials used in this study included 2% nitric acid, paraffin, 10% formalin solution, xylol, immunohistochemical staining reagents, and graded ethanol (70%, 80%, 90%, and 100%) for COL1A1 expression analysis. For the formulation and evaluation of tablets, materials included polyvinylpyrrolidone K30 (PVP K30), microcrystalline cellulose (Avicel PH 102), sodium starch glycolate, magnesium stearate, talc, and distilled water (aquadest).

2.1.3 Ethical approval

This study was conducted following the approval of the Institutional Ethics Committee under approval numbers 580/B.1-KEPK/SA-FKG/VII/2024, 595/B.1-KEPK/SA-FKG/VIII/2024, and 689/B.1-KEPK/SA-FKG/II/2025, Faculty of Dentistry, Universitas Sultan Agung, Semarang, Indonesia.

2.2 Methods

2.2.1 Experimental design

This study employed a post-test only control group design and was divided into three phases of testing. The first test involved analyzing COL1A1 expression in ameloblast cells, with two groups: a control group receiving a normal diet, and a treatment group receiving saltwater fish nanoparticles. The second and third phases were pharmaceutical tests, which included a preformulation test and a physical stability test. These tests were performed according to the three formulation groups, based on the concentration of talc (magnesium silicate hydrate) as a glidant: 1%, 5%, and 10%.

2.2.2 Preparation of saltwater fish nanoparticles

Saltwater fish nanoparticles were prepared from sardines (*Sardinella fimbriata*), splendid pony fish (*Leiognathus splendens*), and tuna (*Euthynnus affinis*). The fish were dried and ground into powder using an Ultra Turrax (IKA, Germany). The powder was mixed with nano chitosan dissolved in 2% acetic acid and sodium tripolyphosphate, then homogenized using a magnetic stirrer (IKA, Germany). The plasma-coupled technique indicated that the powder contained 19.2% CaO, while gas chromatography revealed 74.2 mg/100 mg of Omega-3, 647.6 mg/100 mg of Omega-6, and 16.675.5 mg/100 g of Omega-9 fatty acids were present.

2.2.3 Particle size analysis

The particle size distribution of saltwater fish nanoparticles was determined using a dynamic light scattering (DLS) instrument (Zetasizer, Malvern Panalytical, UK). Measurements were performed at 25 °C in disposable cuvettes, and each sample was analyzed in triplicate. The parameters recorded included the Z-average particle size (d.nm) and the polydispersity index (PDI), which indicates the homogeneity of the sample.

2.2.4 Scanning electron microscopy

Saltwater fish powder and nanoparticles' surface morphology were both examined using scanning electron microscopy (SEM) (JEOL JSM, Japan). The SEM images were obtained under an accelerating voltage of 20 kV, a working distance of 10 mm, and a magnification of 5000x.

2.2.5 Animal experimental

Sixteen female mice (aged 2–3 months) weighing approximately 25 g were acclimatized for 7 days and randomly divided into two groups: control and treatment (n=8 per group). Sample size required six samples per group, with an additional two samples included, plus 2 samples to account for possible dropouts (Lemeshow *et al*., 1990). The treatment group received human equivalent dose conversion of oral saltwater fish nanoparticles at a dose of 2.17 mg twice daily (09:00 and 16:00 Greenwich Mean Time +7) from gestational day 0 to day 18 (Laurence and Bacharach, 1964). The control group received standard diet using boiler started feed (511B, PT Charoen Pokphand Indonesia Tbk., Sidoarjo, East Java, Indonesia) and distilled water *ad libitum* and distilled water *ad libitum*. On day 18 of pregnancy, fetuses were harvested post-anesthesia with chloroform. Fetal head tissues were fixed in 10% formalin, decalcified with 2% nitric acid for 4 h, dehydrated with ascending ethanol concentrations, cleared with xylol, and embedded in paraffin. Tissue sections (3.5 µm thick) were prepared, deparaffinized, rehydrated, and subjected to immunohistochemical staining to assess COL1A1 expression in ameloblasts.

2.2.6 Evaluation of COLIA1 expression

COL1A1 expression was observed under a light microscope at 400× magnification in 5 fields of view per sample. Ameloblast cells exhibiting COL1A1 positivity were identified by light brown staining, and a certified anatomical pathology specialist performed the assessment.

2.2.7 Tablet formulation

Table 1 presents the tablet's composition with the different components' percentage. The tablet formulation began with granule preparation, aiming for a final weight of 500 mg (100% of the formulation), comprising 80% active ingredient (saltwater fish nanoparticles) and 20% excipients. Three formulations were prepared according to the Indonesian Pharmacopoeia VI. The Formulation Codes (F) were assigned to distinguish each formula according to talc concentrations of 1%, 5%, and 10%, used as a glidant. The amount of microcrystalline cellulose (Avicel PH 102) was adjusted to compensate for varying talc contents. The wet granulation method was used for formulation.

Table 1. Composition of saltwater fish nanoparticles' tablet formulation at different talc concentration

Component	Function	Composition (%)		
		F1	F2	F3
Salwater Fish Nanoparticles	Active Ingredients	80	80	80
PVP K30	Binder	2	2	2
Avicel Ph 102	Diluent	11	7	2
Sodium Starch Glycolate	Disintegrant	5	5	5
Magnesium Stearate	Lubricant	1	1	1
Talc	Glidan	1	5	10

2.2.8 Preformulating evaluation

The flow properties of the granules were evaluated by measuring the angle of repose (θ). Approximately 100 mg of granules were placed in a funnel with a top diameter of 12 cm, a height of 4 cm, and a 1 cm bottom diameter. The funnel was initially closed. The cone height (h) and radius (r) of the powder bed were measured, and the angle of repose (θ) was calculated using Formula 1.

$$\theta = tan^{-1}\ \frac{h}{r}. \quad \text{.....(i)}$$

Additionally, the compressibility of the granules was assessed by calculating Carr's Index (CI) and Hausner Ratio (HR) according to the British Pharmacopoeia (Patel *et al*., 2009). Granules were poured into the cylinder until reaching a volume of 100 mL, representing the bulk volume (Vo). The granules were tapped 500× with a tapped density tester to obtain the tapped volume (Vf). Granules were weighed to obtain the mass of granules (Wo). The bulk density (ρ bulk) was calculated using Formula 2, while tapped density (ρ tapped) was calculated using Formula 3. CI and HR were calculated using Formulas 4 and 5, respectively.

ρ bulk = Wo / Vo.....(ii)

ρ tapped = Wo / Vf.....(iii)

CI (%) = 100 × (ρ tapped - ρ bulk) / (ρ tapped).....(iv)

HR = (ρ tapped) / (ρ bulk).....(v)

2.2.9 Tableting process

Granules were compressed into tablets using a single-punch tablet machine under a controlled compression force to ensure a uniform weight of 500 mg for each tablet. The compression pressure was carefully adjusted to maintain consistency across all formulations. Figure 1 tablets were placed in containers according to their formulation code.

2.2.10 Physical stability evaluation

Physical stability was evaluated using three tests, including: i) A hardness test was conducted with a tablet hardness tester, where each tablet was placed centrally, and pressure was applied until fracture. The maximum force before breakage was recorded, ii) Friability test was performed using a friability tester operated at 25 rpm for 4 min (100 rotations), iii) Disintegration test was measured following standard protocols for solid dosage forms, using a disintegration tester.

Figure 1. Saltwater fish nanoparticle tablets.

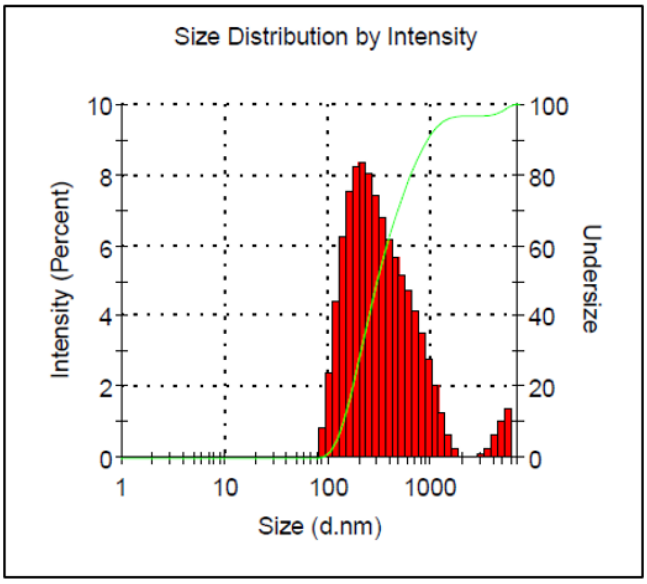

Figure 2. Size distribution of saltwater fish nanoparticles using particles size analyzer.

2.3 Analysis Data

Data from the COL1A1 expression analysis were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test; both tests confirmed that the data met the assumptions for parametric testing. Consequently, an Independent T-test was conducted to compare COL1A1 expression levels between the control and treatment groups at a significance level of $\alpha = 0.05$ and a 95% confidence interval. For the physicochemical evaluations, One-way ANOVA was used to assess differences among groups, followed by Bonferroni post hoc tests. All statistical analyses were performed using SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1 Results

3.1.1 Particle size analysis and scanning electron microscopy of saltwater fish nanoparticles

The PSA analysis revealed that the saltwater fish nanoparticles had an average hydrodynamic diameter (Z-average) of 166.7 nm with a polydispersity index (PDI) of 0.372. Figure 2 demonstrates the particle size distribution curve, with a relatively narrow distribution, indicating that the nanoparticles were within the desired nanometer range and were sufficiently homogeneous for further formulation development. Figure 3 demonstrated irregular rod-like structures with rough surfaces in saltwater fish powder compared with smaller, rounded structures in saltwater fish nanoparticles.

3.1.2 COLIA1 expression in ameloblast cells

Figure 4 illustrates the morphology of ameloblast cells, indicating COL1A1 expression, which was observed under a light microscope at 400× magnification across five fields. These images aim to illustrate the differences in cellular expression or morphology between the two groups, potentially highlighting the impact of nanoparticle supplementation on ameloblast cells. Figure 5 compares COL1A1 expression levels in ameloblast cells between the control group (40.63±13.21) and the saltwater fish nanoparticle treatment group (21.88±13.87). The T-Independent Test revealed that the treatment group of saltwater fish nanoparticle exhibited significantly ($p<0.05$) lower expression levels than the control group, highlighting the potential impact of saltwater fish nanoparticle on ameloblast cellular processes.

3.1.3 Flow properties in preformulation evaluation

Table 2 presents the flow properties of the granules used in tablet formulation, evaluated through the angle of repose (θ), Carr's Index (CI), and Hausner Ratio (HR) for three formulations (F1, F2, and F3). The angle of repose, ranging from approximately 36.97° to 40.07°, indicates good flowability, with lower values suggesting better flow. Carr's Index values between 8.00% and 11.33% reflect excellent flowability and compressibility, while Hausner Ratios close to 1.09–1.13 further confirm good flow characteristics of the granules. These optimal flow properties suggest that the granules are suitable for consistent and uniform tablet production.

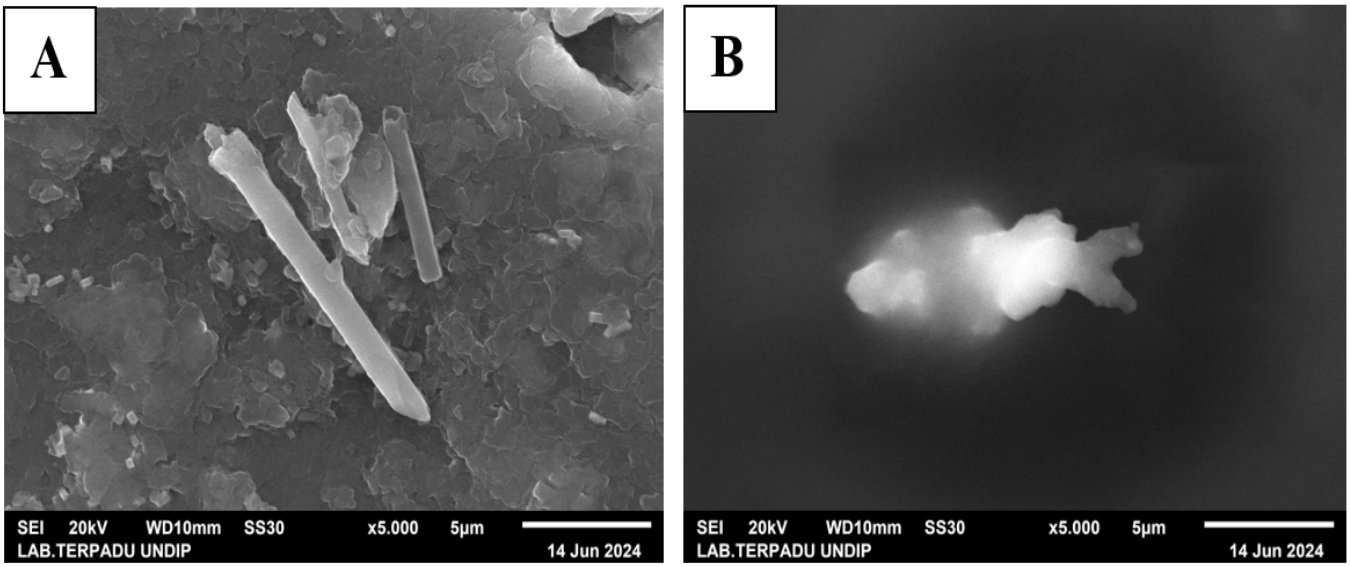

Figure 3. Size morphological structure: (a) Saltwater fish powder (b) Saltwater fish nanoparticles. SEM was done using 5000x magnification.

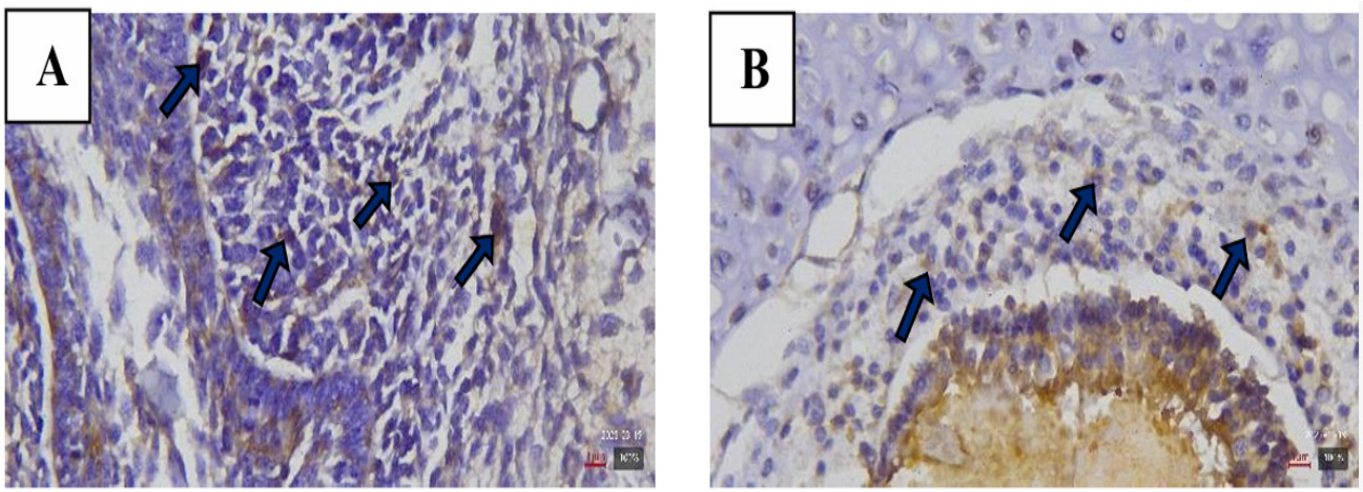

Figure 4. COL1A1expression (black arrow) in ameloblast cells of fetal mice tooth buds: (a) Control group and (b) Treatment group receiving nanoparticle saltwater fish powder. Immunohistochemical staining was used and observed at 400x magnification under a light microscope.

Table 2. Flow properties in preformulation evaluation

Flow Properties	F1	F2	F3
Angle of Repose (°)	40.07 ± 1.89[a]	38.90 ± 0.90[a]	36.97 ±1.24[a]
Carr's Index (%)	11.33 ± 1.53[a]	9.67 ± 0.58[a]	8.00 ± 1.00[b]
Hausner Ratio	1.13 ± 0.02[a]	1.11 ± 0.01[a]	1.09 ± 0.01[b]

Description: Formulation codes were assigned as follows F1 (1% Talc), F2 (5% Talc), and F3 (10% Talc). Values are presented as mean standard deviation (n = 3). Different superscript letters within the same column indicate significant differences between means at $p < 0.05$.

3.1.4 Physical stability evaluation

Table 3 displays the physical stability results of the tablets across three formulations (F1, F2, and F3), focusing on hardness, friability, and disintegration time. The hardness values, ranging from 3.29 to 4.00 kgf, indicate that all tablets possess sufficient mechanical strength to withstand handling. Friability assessment showed minimal weight loss, with values well below the 1% threshold, confirming the tablets' durability during packaging and transport. The disintegration times varied from approximately 1150 to 1482 seconds, exceeding the standard limit of 900 seconds (15 minutes) for uncoated tablets, indicating that all formulations require further modification to meet disintegration requirements. Overall, the results suggest that the tablets possess acceptable physical strength but need optimization to improve disintegration performance.

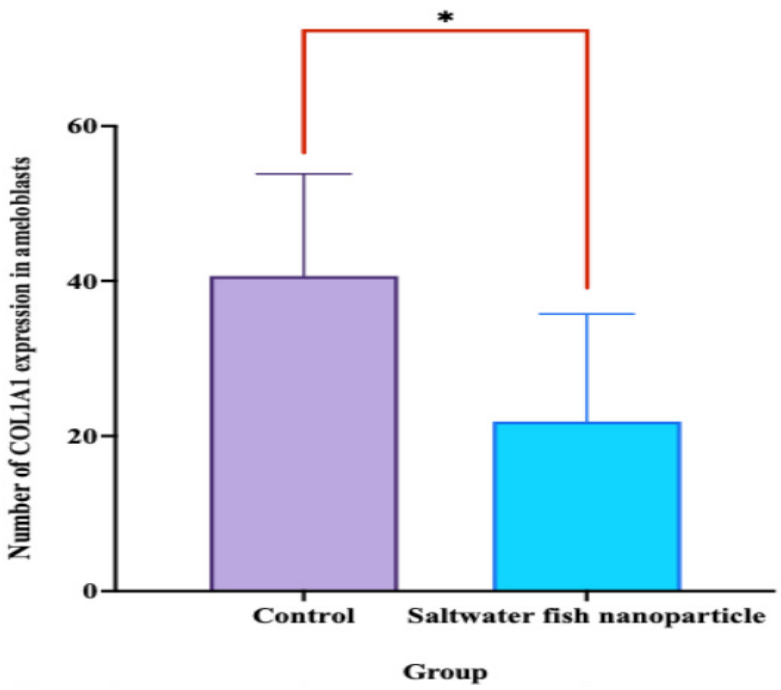

Figure 5. Comparison of COL1A1 expression levels in ameloblast cells between control and treatment groups. The bar graph illustrates the mean expression levels, with error bars indicating standard deviation. * indicates a significant difference in COL1A1 expression as determined by an independent T-test ($p < 0.05$).

Table 3. Physical stability evaluation

Evaluation	F1	F2	F3
Hardness (kgf)	3.29 ± 0.23^{a}	3.58 ± 0.24^{b}	4.00 ± 0.25^{c}
Friability (%)	0.05 ± 0.01^{a}	0.36 ± 0.01^{b}	0.02 ± 0.01^{c}
Disintegration Time (s)	11.50 ± 46.1^{a}	12.66 ± 104.7^{b}	14.82 ± 76.5^{c}

Description: Formulation codes were assigned as follows F1 (1% Talc), F2 (5% Talc), and F3 (10% Talc). Values are presented as mean standard deviation. Different sample sizes were used for hardness (n = 10), friability (n = 5), and disintegration time (n = 6). Different superscript letters within the same column indicate significant differences between means at $p < 0.05$

3.2 Discussion

3.2.1 Particle size analysis and scanning electron microscopy of saltwater fish nanoparticles

The PSA results confirmed that saltwater fish nanoparticles were successfully formulated within the nanometer scale, with an average size of 166.7 nm. A PDI value of 0.372 indicates moderate uniformity, which is acceptable for pharmaceutical nanoparticle systems. Previous studies have suggested that nanoparticles with a size range between 100-200 nm exhibit favorable biological interactions and stability for drug delivery applications (Danaei *et al.,* 2018; Haripriyaa and Suthindhiran, 2023). Therefore, the saltwater fish nanoparticles are a suitable candidate for pharmaceutical formulation and biological evaluation. Morphological structures have been reported as a critical feature in improving the surface area-to-volume ratio of nanoparticles and enhancing their stability and interaction in pharmaceutical systems (Honciuc and Honciuc, 2024; Zhang *et al*., 2022). These findings confirm that saltwater fish powder was effectively modified into a nano scale with favorable morphological properties for biomedical applications.

3.2.2 COLIA1 expression in ameloblast cells

This study demonstrated that the administration of saltwater fish nanoparticles significantly reduced COL1A1 expression in ameloblast cells compared to normal feeding, which represents a physiological transition toward enamel maturation, during which collagen expression gradually declines as mineral deposition and enamel hardening progress. The expression of COL1A1 by ameloblasts is more prominent during the enameloid deposition and maturation stages. As amelogenesis progresses, it decreases and in due course of time it disappears. The COL1A1 gene encodes the pro-α1(I) chain of type I collagen, which is a major structural protein in the extracellular matrix (Hou *et al*., 2021). In the context of dental tissue, COL1A1 is expressed in newly differentiated odontoblasts responsible for secreting predentin, as well as in fully differentiated odontoblasts. The expression pattern of COL1A1 closely correlates with dentin sialophosphoprotein (DSPP), another critical marker involved in dentin mineralisation. These observations provide direct evidence that the dental pulp contains competent progenitor cells capable of differentiating into odontoblast-like cells that express high levels of COL1A1 and DSPP, subsequently secreting tubular reparative dentin necessary for tooth repair and regeneration (Braut *et al*., 2003).

Furthermore, COL1A1 is a temporal transcriptome that peaks during the early stages of odontoblast differentiation. Type I collagen, formed by the COL1A1 gene product, constitutes one of the first extracellular matrix components expressed during dentinogenesis. It provides a scaffold that guides and supports controlled calcium phosphate deposition, a critical step in mineralised tissue formation (Teti *et al*., 2013). During embryonic tooth organogenesis, the inductive ectomesenchyme develops at the sites of future permanent teeth. In this context, both DSPP and COL1A1 are key ECM components essential for proper dentin formation. Although some downregulation of COL1A1 may occur in vitro under certain conditions, it remains relatively highly expressed, ensuring the structural integrity of developing dentin tissue (Rosowski *et al*., 2019).

Calcium-based interventions, including genetic modifications and nutritional supplementation such as chitosan-calcium and omega-3 nanoparticles, have shown promising potential in modulating histological processes within dental tissues, especially affecting ameloblast formation and function (Said *et al*., 2020). Notably, COL1A1 expression is tightly regulated through multiple signalling pathways, and disruptions in these pathways can impair odontoblast and ameloblast differentiation. The observed reduction in COL1A1 expression following nanoparticle administration may be attributed to interference with these regulatory mechanisms, thereby impacting ameloblast proliferation, differentiation, and function during amelogenesis. This COL1A expression decreases after metamorphosis, indicating a transition from enameloid to enamel formation (Assaraf-Weill *et al*., 2014).

Additionally, the TGF-β1 signalling pathway plays a pivotal role in amelogenesis by regulating cellular proliferation, differentiation, and apoptosis of ameloblasts, as well as the expression of enamel matrix proteins such as AMELX, AMBN, and ENAM, and proteolytic enzymes like MMP20 and KLK4 involved in enamel maturation. The involvement of TGF-β1 in regulating key components during enamel formation supports the present findings, indicating that administration of calcium, omega-3, and chitosan nanoparticles may reduce ameloblast numbers and COL1A1 expression, consequently affecting ameloblast biological activity, differentiation, and enamel mineralization during pregnancy (Ma *et al*., 2025). Previous studies have also revealed that a diet supplemented with nano calcium and vitamin D accelerates tooth development and eruption (Sitosari *et al*., 2020).

Regarding the potential effects of saltwater fish nanoparticles specifically, these particles are rich in bioactive nutrients such as omega-3 fatty acids, peptides, and minerals, which have been shown to modulate cellular signalling pathways involved in tissue regeneration and gene expression (Christiono *et al*., 2022; Christiono *et al*., 2023a). In mice, sodium-rich compounds from saltwater fish may influence collagen synthesis pathways, either by providing essential precursors or by altering cellular signalling cascades that govern COL1A1 expression. This could result in either upregulation or downregulation, depending on the specific composition and dosage, thereby impacting the structural composition and repair capabilities of dental tissues, including ameloblast function and dentin formation. Further research is necessary to elucidate the precise molecular mechanisms and the long-term implications of saltwater fish nanoparticles on dental tissue regeneration and gene regulation in vivo.

3.2.3 Flow properties of saltwater nanoparticle granules were affected by talc concentration

The results indicated that increasing talc concentration led to a decrease in the angle of repose, Carr index, and Hausner ratio, reflecting improved flow characteristics. Flowability is a critical parameter in drug preformulation, particularly for tablet manufacturing, as it influences uniformity in tablet weight and ensures consistent physicochemical properties (Chendo *et al*., 2023). The observed reduction in the angle of repose with higher talc concentrations can be attributed to talc's lubricating and glidant properties, which act via a "ball bearing" effect. Talc forms a monolayer on particle surfaces, thereby decreasing intergranular cohesion and facilitating smoother flow (Shah *et al*., 2021). Additionally, increasing talc concentration enhances particle sphericity and aggregate size, further improving flow characteristics (Varia *et al*., 2022).

Comparison studies have shown talc's superior flow properties relative to other excipients. It was reported that talc displayed better Carr index and Hausner ratio values compared to microcrystalline cellulose (MCC) and bentonite (Eraga *et al*., 2015). Similarly, it was observed that talc exhibited superior flow indices compared to cottonseed addition (CSD) or their mixtures (Apeji and Olowosulu, 2020). It was also found that increasing talc concentration (by 20 mg per formula) improved both the Carr index and Hausner ratio, reinforcing talc's role in enhancing flowability (Dinesh and Mutahar, 2009).

3.2.4 Physical stability of the saltwater fish nanoparticle tablet was affected by different talc concentrations

The physical stability assessment revealed significant differences in tablet hardness, friability, and disintegration time with varying talc concentrations (1%, 5%, and 10%). Tablet hardness influences key quality attributes such as disintegration and dissolution rates; heavier tablets typically dissolve more slowly, whereas overly hard tablets can delay disintegration and reduce bioavailability, while fragile tablets are prone to breakage during handling (Siraj *et al*., 2025). An ideal hardness range is typically between 4–8 kgf. In this study, F1 (3.297 kgf) and F2 (3.557 kgf) did not meet this criterion, whereas F3 (4.001 kgf) did, making the latter the optimal formulation in terms of mechanical strength.

Friability testing evaluates a tablet's resistance to mechanical stress. A friability value below 1% indicates acceptable resistance, minimising the loss of active ingredient during handling and transportation (Zhao *et al*., 2022). All formulations F1 (0.0542%), F2 (0.3660%), and F3 (0.0190%) met this standard, with F3 demonstrating the lowest friability and thus the greatest mechanical stability.

Disintegration time is crucial for ensuring the rapid onset of action, with uncoated tablets required to disintegrate within ≤15 minutes in gastrointestinal fluid (Al-Gousous and Langguth, 2014). In this study, all formulations, F1 (19 min 9 sec), F2 (21 min 1 sec), and F3 (24 min 42 sec) failed to meet this criterion, indicating prolonged disintegration potentially due to the influence of talc as a lubricant, which can hinder water penetration.

Talc's role as a lubricant reduces friability and increases tablet hardness by strengthening granule cohesion and reducing intergranular friction. It also promotes better flow of particles, allowing for optimal die filling and fewer air gaps during compression. Higher talc content tends to increase granule moisture, further enhancing hardness and reducing friability. However, this increased moisture content, along with talc's hydrophobic nature, can prolong disintegration and slow dissolution by hindering solvent penetration (Fu *et al*., 2020).

Increased talc as a lubricant excipient enhances tablet hardness and decreases friability; however, it also prolongs disintegration time (Alita and Suprapto, 2023; Kuno *et al*., 2008). Based on the comprehensive tablet physical tests evaluating hardness, friability and

disintegration, Formulation 3 was identified as the best overall. It met the hardness and friability standards but did not satisfy the disintegration time requirement.

4. Conclusion

Saltwater fish nanoparticles significantly decreased COL1A1 expression in ameloblast cells, suggesting their potential influence on fetal tooth development in mice. Furthermore, increasing talc concentration improved flow properties, reducing the angle of repose, Carr index, and Hausner ratio, and resulted in tablets with greater hardness and lower friability, although higher talc levels also contributed to prolonged disintegration times. The improved physical stability of the tablets supports the potential for developing a standardized oral delivery system for saltwater fish nanoparticles.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author is as follows: SC, IRH, and SA collected the data and drafted the manuscript. ADC, ROPA and AFS collected the data and reviewed the citation. SS designed the figure, table, and review and revised the manuscript. All authors discussed the results and contributed to the preparation of the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

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