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Research article

Study of the Potential Application of Hydroxyapatite (HAPt) from Cattle Bone in the Tooth Demineralization Process

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Abstract

One of the animal by-products that includes most inorganic compounds, specifically calcium and phosphorus, is cattle bone. In this study, the possible use of HAPt derived from cattle bone waste to counter tooth demineralization was assessed. A 5x5 unidirectional pattern and a completely randomized design were used in the experimental design of the investigation. Five different types of soaking material treatments were used: S0 = acetate buffer (AB) (control); S_1 = AB+NaF 10 mg/L (w/v); S_2 = AB+HAPt 25 mg/L (w/v); S_3 = AB+HAPt 50 mg/L (w/v); and S_4 = AB+HAPt 100 mg/L (w/v). The following periods were observed: 3, 6, 9, 24, and 48 h. The findings demonstrated that the percentage of tooth mass loss during the soaking process was significantly ($p<0.01$) impacted by variations in the kind of material and HAPt level. Compared to other treatments, HAPt at 50 and 100 mg/L had the best ability to slow down the rate at which teeth were demineralizing. The tooth wall segment was considerably protected from fracture during the demineralization process in AB solution pH 5, 1 M, by the application of HAPt from cattle bone at 100 mg/L. The study's overall findings demonstrated that HAPt, which is made from cattle bone waste from the foreleg (*Os metacarpus*), may be used in dental treatment to slow down the rate of tooth demineralization.

Keywords: hydroxyapatite (HAPt); cattle bone; demineralization; acetate buffer; tooth

1. Introduction

The tooth is one of the tools that support the human digestive system. The majority of people have caries, a form of tooth decay. Dental cavities may affect 90% of youngsters. This indicates that carries are a serious problem for teeth and oral health (Van Chuyen et al., 2021). The caries process in teeth can demineralize, cavitate, and damage the hard

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structure of the teeth due to pollution and microbial activity (Rathee & Sapra, 2023). Microorganisms (bacteria) will change sugar groups (glucose, fructose, and sucrose) into acidic compounds through a process called glycolysis. The pH of the mouth rapidly drops as a result of this circumstance, which increases the flow of hydrogen ions (H^+). These hydrogen ions can damage the dental enamel hydroxyapatite structure (Burne & Marquis, 2000). When hydrogen ions bind to phosphate ions in hydroxyapatite, HPO43- is produced. Because these ions are out of balance with normal hydroxyapatite, some of the hydroxyapatite crystals in tooth enamel disintegrate as a result of this condition. As a result, the tooth structure becomes brittle. This process can potentially be controlled or stopped with remineralization techniques. Such process can include restoring the pH of the mouth to neutral and providing adequate calcium and phosphate because these mineral elements have been lost (Amaechi & Higham, 2001). To maintain the state of the teeth, the demineralization process needs to be halted and reduced.

Dental plaque buildup on the tooth's upper border is typically the cause of the secondary caries process. Cariogenic bacteria release acids and enzymes during fermentation, which is why this happens (Delaviz et al., 2014; Jiao et al., 2019). The space between teeth is harmed by the enzymes that bacteria create. This also increases the risk of dental cavities and can be harmful. Consequently, the tooth restoration procedure should offer long-term protection (Carvalho & Manso, 2016). One biomaterial that has garnered much interest from researchers is hydroxyapatite (HAPt), which is used in dental implant coatings, soft tissue engineering, periodontal defect repair, and bone tissue reconstruction (Jimbo et al., 2012). It is possible to use the calcium ion content of beef bones as a raw material to make HAPt. HAPt can be used as a chemical to stop teeth from demineralizing, among other things. Some of the HAPt compounds in tooth enamel may be eliminated during the demineralization process.

Researching and developing the potential of HAPt from cattle bone waste is intriguing. These researchers' considerations are inextricably linked to HAPt's special characteristics. One kind of material with excellent biocompatibility and a strong affinity for biopolymers is synthetic HAPt (Kantharia et al., 2014). It has been demonstrated that HAPt is osteoconductive, biocompatible, and capable of promoting bone formation and osteoblast differentiation. Applications in dentistry have grown, particularly in soft tissue engineering, periodontal therapy, dental implant coatings, filler restorative materials, and bone tissue regeneration (Kattimani et al., 2014; Kasaj et al., 2012; Jimbo et al., 2012). HAPt has been effectively synthesized from the front foreleg of cattle bones (*Os metacarpus*). This endeavor aimed to investigate the possibilities of animal by-products that have not yet been used. In 2021, 67,993.6 tons of cattle bone debris were produced in Indonesia. As more cattle are killed for animal feed (meat), the amount of cattle bone waste produced will also continue to rise (Said et al., 2024). The application of HAPt in tooth protection has been evolving steadily, particularly in the demineralization of the tooth's dentin layer. This process is associated with the development of dentin sensitivity. When dentin is exposed to chemical, thermal, tactile, or osmotic stimuli, it can produce a tingling sensation known as hypersensitivity (Gillam, 2015). The reduction of tooth sensitivity is linked to the application of nano-hydroxyapatite. Since nano-hydroxyapatite is made of calcium and phosphate, it is believed to help seal dentin tubules. Hydroxyapatite is also supersaturated in human saliva in the oral cavity (Gopinath et al., 2015). Therefore, this study aimed to assess how the administration of HAPt from raw cattle bone materials in an acid buffer soaking solution could affect the demineralization process of teeth.

2. Materials and Methods

2.1 Materials

The primary source of material for this investigation was the bone of three-year-old Bali cattle (*Bos sondaicus*). The forelegs of Bali cattle (*Os metacarpus*) provided bone by-product material. The results of earlier research investigations indicated that the front foreleg bone of cattle (*Os metacarpus*) was one of the greatest raw material features for generating HAPt (Said et al., 2024), which was why samples were taken in this part (Figure 1).

The main materials were obtained from the slaughterhouse unit in Tamangapa village, Gowa regency, Indonesia. Human tooth samples were obtained from the Dental and Oral Hospital, Hasanuddin University, Makassar, Indonesia. The supporting materials were: distilled water, $(NH_4)_2HPO_4$ 0.3 M, glacial CH_3COOH 100%, $CH_3COONa.3H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$, NaF, Whatman No.42 filter paper, aluminum foil and distilled water. The equipment used in the production and testing process were: furnace (*Furnace 6000*), UV-Vis- spectrophotometer (*Shimadzu 6105 and Hitachi Flexsem 100*), vacuum pump (*Sargen-Welch Co. model 1400*), oven (*BMT Inconel V-LSIS-B2V/IC55*), analytical balance (*OHAUS PA214C*), magnetic stirrer (*Stuart SD162*), thermometer, desiccator, stopwatch, beaker glass, Erlenmeyer flask, measuring cup, and volume pipette.

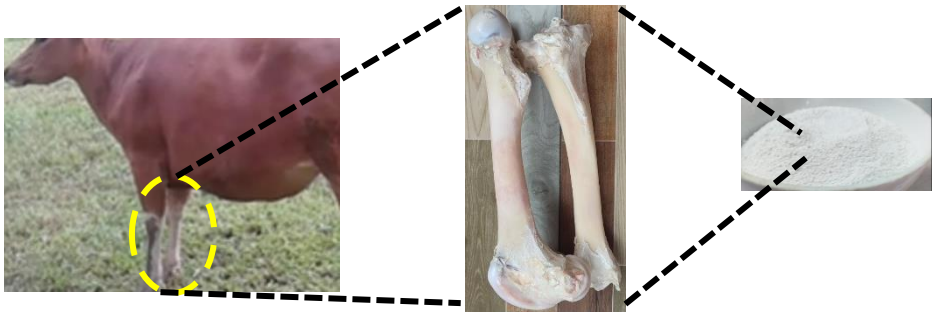

Figure 1. Section for taking bone samples from foreleg (*Os metacarpus*) of Bali cattle

2.2 Methods

2.2.1 Hydroxyapatite (HAPt) production process

After being treated, cleaned, and weighed, 500 g of cattle bone debris was obtained. The production process for HAPt was conducted using the methodology developed by Khiri et al. (2016). Three steps were involved in manufacturing HAPt from cattle bone: calcination, precipitation, and sintering.

Stage 1. Calcination process. Calcination is the first step in the formation of hydroxyapatite (HAPt) ($Ca_{10}(PO_4)_6(OH)_2$). The calcination process was initiated at 900ºC using a furnace for 5 h; Stage 2. Precipitation process. The material was finely milled into 45 µm-long particles and 50 mL of distilled water at 41°C was added to the bone sample. After that, the mixture was agitated for 5 min at 800 rpm using a magnetic stirrer. Then, 50 mL of a 0.3 M phosphoric acid (HPO_4) solution was added to the $Ca(OH)_2$ suspension at 15–20 drops per minute. A magnetic stirrer was used to keep the pH of the solution

constant at 8. Ammonium hydroxide (NH_4OH) solution was added to the mixture until the reaction was complete. After that, the resulting solution was kept at room temperature for a full day. Whatman filter paper no. 42 was then used to filter the white precipitate that had formed. After that, the precipitate was dried at 95ºC for 5 h. The sintering technique was then used to continue the process outcomes; Stage 3. Sintering process. After that, the bone powder product was sintered for 1 h at 800ºC in a furnace. After that, the bone powder was cooled to room temperature in a furnace. Following the sintering, the hydroxyapatite (HAPt) products were produced. As the human tooth demineralizes, this substance became a protective layer.

2.2.2 Preparation of tooth samples

To meet the requirements of the five treatments (S_0, S_1, S_2, S_3, and S_4), 25 tooth samples (samples in combination with premolars, molars, canines, and incisors) were prepared with five replications. Teeth samples were taken from Hasanuddin University's Dental and Oral Hospital in Makassar City, Indonesia. The samples were then rinsed under running water after using a brush to remove any remaining dirt. The samples were weighed after drying at room temperature to ascertain the initial weight. To facilitate the soaking process, 25 beakers of 250 mL capacity were randomly filled with tooth samples. All 25 beakers were filled with 150 mL of acetate buffer (AB) 1 M, pH 5, as a soaking solution. S_0 was a negative control consisting only of AB; S_1 was a positive control consisting of AB+NaF 10 mg/L (w/v); S_2 was AB+HAPt 25 mg/L (w/v); S_3 was AB+HAPt 50 mg/L (w/v); and S_4 was AB+HAPt 100 mg/L (w/v).

2.2.3 Research design and statistical analysis

The study utilized a 5 x 5 pattern and a completely randomized design (CRD). Five treatments in all were used. Five repetitions of each treatment were made. S_0 was the negative (-) control, S_1 was the positive (+) control, S_2 was acetate buffer plus HAPt 25 mg/L, S_3 was acetate buffer plus HAPt 50 mg/L, and S4 was acetate buffer plus HAPt 100 mg/L. While the microstructure was examined descriptively, ANOVA was used to investigate the variations in the percentage of mass loss and phosphate ion levels throughout the soaking process. Duncan's Multiple Range Test (DMRT) was then used to check for significant differences at a 5% level for treatments that showed a significant effect (Steel & Torrie 1960).

2.2.4 Parameters and data analysis

Reduction of tooth mass (RTM): The percentage of tooth mass was calculated by measuring the tooth's mass (g) before and after the immersion process. The equation for calculating the reduction of tooth mass (RTM) was: (RTM) % = 100%-M%, where the percentage of the mass of tooth (M)% = a(g) – b(g)/a(g) x 100%; a = mass (g) of the tooth before immersion; b = mass of the tooth after immersion (g).

Evaluation of levels of phosphate ion: The acetate buffer (AB) soaking material's basic solution was prepared at a concentration of 1 M and conditioned at pH 5. The solution's primary component was made entirely of glacial acetic acid. To produce a color mark in the titration procedure, a mixture of $(NH_4)_6Mo_7O_{24}.4H_2O$, NH_4VO_3, and concentrated HNO_3 was used to create the color reagent solution. A five concentration series—12.5 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, and 200 mg/L—were used to create the standard phosphate solution from NH_42HPO_4 from the 1000 mg/L stock solution. For all

treatments (five treatments with five replications), 150 mL of 1 M acetate buffer solution, pH 5.0, was added to 25 beakers based on the AB solutions prepared. There was a negative control (only AB solution) (S_0), a positive control treatment (S_1) that had NaF 10 mg/L solution, and three treatments (S_2, S_3, and S_4) that contained HAPt solution with concentration variations of 25 mg/L, 50 mg/L, and 100 mg/L. The teeth were soaked for 3, 6, 9, 24, and 48 h. The solutions were taken at different five-times series, and a UV-Vis spectrophotometer set to 360 ηm wavelength was used to observe the results. Figure 2 clearly illustrates the treatment model for the demineralization process of dental samples in an acetate buffer (AB) solution.

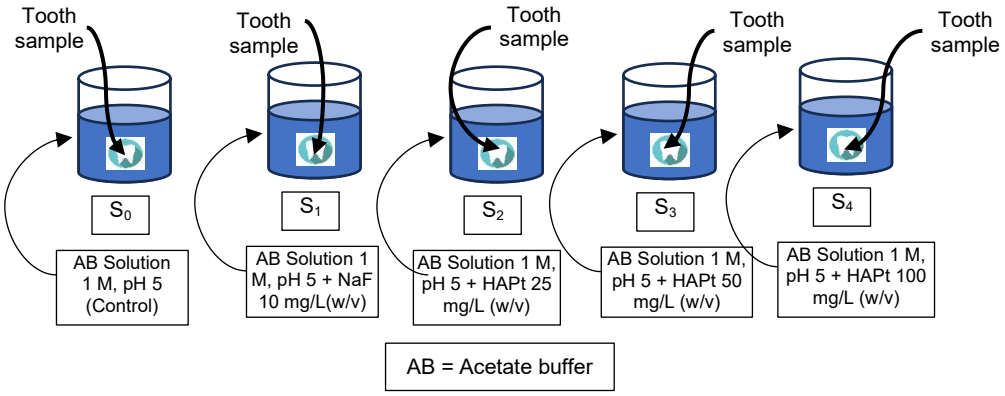

Figure 2. Illustration of the treatment model for the demineralization process of tooth samples in acetate buffer (AB) solution

Morphology and microstructure of tooth by SEM: To enhance conductivity and picture quality, conductive gold metal was applied to the teeth samples after they had been cleaned and prepped. After that, each sample was put into the SEM's vacuum chamber, where an electron beam was directed and moved across the surface. A detector then captured the resultant electrons to create a detailed surface composition and topography picture. After that, the picture readings were interpreted.

3. Results and Discussion

3.1 Reduction of tooth mass (RTM)

When a tooth was soaked in acetate buffer solution, its RTM reveals several dental masses that melt and disappear. The process of tooth demineralization is linked to the loss of tooth mass. The loss of different mineral ions from dental enamel, which is mainly composed of hydroxyapatite (HAPt) crystals made of $Ca_{10}(PO_4)_6(OH)_2$, is known as demineralization. The degree of HAPt porosity can also affect the binding response between teeth and HAPt. Compared to HAPt with a dense structure, porous HAPt is more readily absorbed and more osteoconductive (Chang et al., 2000). Figure 3 presents all of the data of RTM in teeth.

The statistical analysis of the data in Figure 3 indicates that the loss mass value of tooth samples throughout the immersion process was significantly impacted (p<0.01) by the variation in the usage of HAPt in acetate buffer solution. One of HAPt's roles is to stop interactions with acidic substances from removing calcium ion components from teeth. The graph shows that, in comparison to the control treatment (S_0), the HAPt treatments (S_2, S_3,

and S_4) were substantially more effective at preventing the removal of tooth components after immersion in acidic solutions (acetate buffer). NaF, a chemical with characteristics similar to HAPt, was used in Treatment S_1 as a positive control. The F element in NaF and Na in HAp have better preventive properties against tooth decay, so they are often applied in toothpaste. Both of these materials were found to prevent the demineralization process due to the influence of acid (Karlinsey et al., 2011). Treatment S_0 had the highest degree of tooth mass loss (37.65%±0.19) compared to the other four treatments (S_1, S_2, S_3, and S_4), which were 3.41%±0.01; 5.04%±0.01; 3.21%±0.01 and 2.87%±0.01, respectively, according to the observation data. Treatment S_4 (acetate buffer + HAPt 100 mg/L) had the lowest mass loss value, while S_2 (acetate buffer + HAPt 25 mg/L) and S_3 (acetate buffer + HAPt 50 mg/L) had the second-largest mass loss values. A low mass loss value indicates the lowest stage of the tooth demineralization process. The S_4 treatment had the lowest mass loss, suggesting that administering HAPt at a concentration of 100 mg/L can offer the highest level of protection compared to other treatments. In theory, the tooth's structure is made up of biological and inorganic elements. According to Kim et al. (2010), collagen molecules or other non-collagen proteins make up the organic components of teeth. Teeth include inorganic components in addition to biological ones. Calcium phosphate, or HAPt, is one of them. The osteoconductive and bioconductive properties of HAPt are also found in teeth. The loss of Ca^{2+} ions from teeth affects the pH value (Zawacki et al., 1990). This raises the possibility that the demineralization process is intimately linked to the solvent's state as an acid with a low pH.

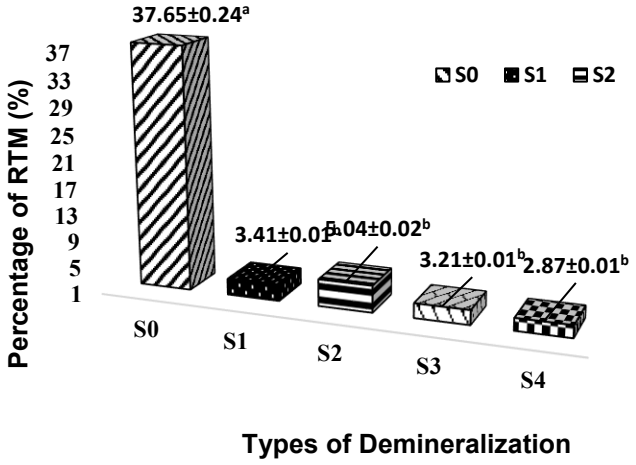

Figure 3. Reduction of tooth mass (RTM)(%) values in teeth soaked in 1 M, pH-5 of AB solution for 48 h with HAPt treatment
Note: Acetate buffer (AB); S_0 = (AB)(control); S_1=AB + NaF 10 mg/L; S_2=AB + HAPt 25 mg/L; S_3=AB + HAPt 50 mg/L; S_4=AB+HAPt 100 mg/L. ^{a,b} Different treatment superscripts indicate significant differences (p<0.01).

3.2 Levels of phosphate ion

The profile of phosphate ion levels in the acetate buffer soaking solution during the soaking process is completely presented in Table 1.

Table 1. Phosphate ion level profile of demineralized tooth samples in acetate buffer during the soaking process

Types of Demineralization	Demineralization Times (h)				
	3	6	9	24	48
S_0	0.223±0.04[a]	0.321±0.01[b]	0.292±0.02[d]	0.271±0.03[a]	0.196±0.03[a]
S_1	0.263±0.03[a]	0.314±0.02[a]	0.280±0.03[b]	0.254±0.03[a]	0.181±0.04[a]
S_2	0.320±0.00[b]	0.327±0.01[c]	0.270±0.03[a]	0.493±0.05[b]	0.408±0.03[b]
S_3	0.328±0.00[c]	0.337±0.00[d]	0.291±0.03[d]	0.524±0.05[c]	0.461±0.07[c]
S_4	0.348±0.15[d]	0.327±0.15[c]	0.285±0.12[c]	0.685±0.29[d]	0.602±0.25[d]

Note : Acetate buffer (AB) S_0=(AB)(control); S_1=AB + NaF 10 mg/L(w/v); S_2=AB + HAPt 25 mg/L(w/v); S_3 = AB + HAPt 50 mg/L(w/v); S_4=AB+HAPt 100 mg/L(w/v). [a,b,c,d] Different treatment superscripts indicate significant differences (p<0.01).

Table 1 indicates that throughout the soaking process in AB solution with a pH of 5 and a concentration of 1 M, the variations in the kinds and concentrations of HAPt materials had a highly significant (p<0.01) impact on the phosphate ion level. Phosphate ion levels in the soaking solution for the five treatment types ranged from 0.223 to 0.348 mg/L during the first 3 h of soaking conditions. In contrast to the NaF 10 mg/L solution (S1), which was a positive (+) control, and the HAPt solution in treatments S_2 (25 mg/L), S_3 (50 mg/L), and S_4 (100 mg/L), where each phosphate ion content was 0.233 mg/L; 0.263 mg/L; 0.320 mg/L; 0.328 mg/L, and 0.348 mg/L, the data in the Table 1 show that the level of phosphate ions in the solution without HAPt (S_0) (0 mg/L) as a negative (-) control increased. These findings suggest that the demineralization process was proceeding at a slower pace. Hydroxyapatite (HAPt) is the main mineral that makes up tooth enamel and dentin. Its stable crystal structure and chemical composition similar to tooth minerals make it very important in maintaining the strength and integrity of teeth. Phosphate ion dissolution occurs when tooth minerals, especially hydroxyapatite, undergo a demineralization process due to acids produced by AB solvents. Phosphate ions (PO_4^{3-}) and calcium ions (Ca^{2+}) are released from the hydroxyapatite crystal structure. The HAPt provided can act as a stable source of phosphate and calcium ions, thus balancing the ions dissolution by acids. With the supply of ions from HAPt, the dissolution of phosphate ions from the teeth is reduced because the solution around the teeth becomes saturated or nearly saturated with these ions. This can reduce the rate of demineralization and slow down the occurrence of tooth decay. The application of HAPt in research related to tooth dissolution continues to increase and develop. Acetate buffer is widely used as a buffer solution. After immersion for 6 h, more observations were made. Phosphate ion levels in the solution were analyzed, and the results nearly matched the initial measurements (3 h). The HAPt-containing solutions (S_2, S_3, and S_4) had greater phosphate ion levels (0.327, 0.337, and 0.327 mg/L) than the control solutions (S_0 and S_1) (0.321 and 0.314 mg/L). Additional analysis was conducted after 9 h of soaking. Increasing the soaking time from 3 h to 6 h tended to increase the phosphate ion level significantly except in treatment S_4. In the initial study of this study, a series of immersion time treatments were carried out. The immersion times were 3 h; 6; 9 h; 12 h and 24 h. As a result, for the immersion process for 3; 6; 9 12 h, it turned out that all treatments did not show any significant dissolution process. At 24 h of immersion, the dissolution process only occurred for the treatments S0; S1; S2; S3 and S4. For this reason, we only chose a 24-h immersion time as an effective immersion time for the HAPt protection process on teeth. The range of the phosphate ion levels was 0.270-0.292 mg/L. Changes in the phosphate ion levels in the acetate buffer solution at 24- and 48-h immersions displayed a highly significant characteristic. In 24-h and 48-h immersions,

the impact of changes in the immersion process was visible compared to 3, 6, and 9-h immersions. The level of phosphate ions detected in the solution indicated the dissolution process had occurred. Teeth soaked in acetate buffer undergo a dissolution process. This is because the acidic nature of the buffer lowers the pH and causes H^+ ions to attack the hydroxyapatite structure in the teeth. As a result, this process breaks down mineral bonds and releases calcium and phosphate ions into the solution. Acetate ions also help accelerate dissolution by binding calcium ions. Therefore, the released phosphate ions are an indicator that tooth mineral dissolution is taking place. Table 1 illustrates that, in contrast to the negative (S_0) and positive (S_1) controls, the phosphate ion levels for solutions containing HAPt (S_2, S_3, and S_4) displayed the highest values. The average S_2, S_3, and S_4 values during 24-h observations were 0.493, 0.524, and 0.685 mg/L, respectively, but the values for treatments S_0 and S_1 were lower, at 0.271 and 0.254 mg/L, respectively.

In comparison to the S_0 and S_1 treatments, which were 0.196 and 0.181 mg/L, respectively, the profile of the acetate buffer soaking solution for a 48-h soaking time acquired values (S_2, S_3, and S_4) were likewise higher, at 0.408, 0.461, and 0.602 mg/L. The S_2, S_3, and S_4 treatments were shown to be generally effective in preventing tooth demineralization based on observations made during a variety of soaking times. The rising concentrations of phosphate ions from HAPt in the acetate buffer solution demonstrate this. The demineralization process can occur in acidic environments, particularly in the outermost layer of teeth. The solubility of cations in HAPt is the cause of this. Dental cavities may result from this condition (Dawes, 2003). Organic materials in teeth, such as collagen matrix and growth factors, may become visible due to demineralization. This significantly improves the conditions for the development of new tooth material. Compared to dentin, which undergoes calcification, the portion of the tooth that experiences decalcification is more active in the bone production process (Murata et al., 2011). The process of mineralization is ongoing. When inorganic materials precipitate on an organic matrix, this process takes place. Developing hard connective tissues, including bone, dentin, and cementum, is a normal biological process. Calcium phosphate crystals are arranged on a scaffold made of collagen fibrils. Teeth are mainly composed of a mineral chemically known as calcium phosphate hydroxide. Phosphate ions are the main component of this mineral. When teeth undergo dissolution (demineralization), phosphate ions are released from the mineral structure and enter the solution. Acetate buffer (AB) is principally used to maintain the pH of the solution at a certain level that is relevant to the conditions of the human mouth, especially in the acidic pH range that can cause tooth dissolution. AB can help simulate the acidic environment that causes the demineralization process. Measuring phosphate ions in AB solution allows researchers to determine how much tooth mineral has been dissolved. The higher the concentration of phosphate ions detected, the greater the level of tooth dissolution or demineralization that occurs. By measuring phosphate ions, researchers can study the kinetics of dissolution and chemical interactions between tooth minerals and the acidic environment. This helps in understanding the demineralization process in more depth. Phosphate ion measurements are also used to assess the effectiveness of various dental protection materials or methods, such as HAPt treatment, which can inhibit mineral dissolution. If the measured phosphate ions are low, it means that the protection is effective. In its implementation, measuring phosphate ion levels in research is related to several benefits: (1) Development of dental care products: Phosphate ion measurement data helps in the formulation of toothpaste, mouthwash, or restorative materials that can prevent demineralization. (2) Understanding caries risk: By knowing the level of dissolution, the risk of caries formation can be predicted and prevented early. (3) Basic and applied research: Providing a strong scientific basis for further studies on dental health and chemical interactions in the mouth.

3.3 Morphological and microstructural analysis

The description of the differences in morphology and microstructure of teeth without HAPt administration (as control) and with teeth given HAPt in acetate buffer solutions is presented in full in Figures 4a, 4b and 4c.

Figures 4a; 4b and 4c are representations of SEM images of teeth that were treated with negative control (without HAPt/only AB); positive control (NaF 10 mg/L) (w/v); and HAPt treatment, respectively. Treatment S_4 (100 mg/L) (w/v) was the treatment with the highest dose. The shape and microstructure of teeth treated with HAPt in acetate buffer solution and teeth not treated with HAPt are contrasted in Figure 4. The tooth morphology after 48 h of soaking in acetate buffer solution without HAPt acting, which was a negative (-) control, is shown in Figure 4(a). The descriptive analysis demonstrates that the tooth wall surface developed cracks during the soaking procedure, as shown in Figure 4(a). This was probably due to the acidic nature of acetate buffer, which had the potential to disintegrate the tooth layer. As a positive (+) control, the morphology of teeth treated with a coating of sodium fluoride (NaF) at a concentration of 10 mg/L is shown in Figure 4(b). The tooth in the photo is still fractured after being soaked in AB solution. The analysis demonstrates that a NaF 10 mg/L (w/v) solution was unable to offer complete tooth protection. This outcome differs significantly from that shown in Figure 4c. Figure 4c shows that teeth treated with an additional 100 mg/L (w/v) of HAPt in AB solution could prevent cracks. In theory, the demineralization process can make tooth materials more capable of inducing osteogenesis. Exposing organic matter to the tooth surface can increase porosity and surface area (Park et al., 2015). The pH of saliva affects tooth mineralization. More hydrogen ions will be present in saliva with a lower pH, which can disintegrate enamel crystals and harm the hydroxyapatite bonding in teeth. The use of hydroxyapatite obtained from cow bone waste has great potential and promising prospects in various fields, especially in the biomedical, environmental, and industrial fields. HAPt from bovine bone has a chemical composition and crystal structure that is very similar to human bone, making it very compatible for medical applications. HAPt has high biocompatibility and bioactive properties, so it can be accepted by the body without causing rejection reactions.

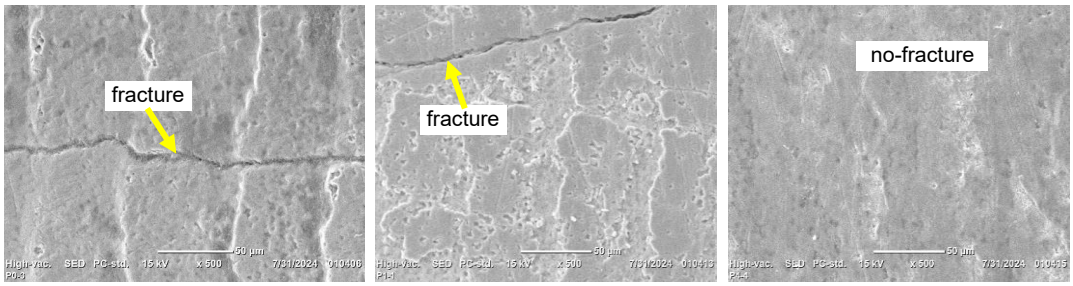

Figure 4. Morphology and microstructure of tooth surfaces after soaking in pH 5, 1M acetate buffer solution for 48 h using a scanning electron microscope (SEM);
Note: (4a) = tooth without HAPt as negative (-) control; (4b) = tooth with NaF 10 mg/L(w/v) treatment as positive (+) control; (4c) = tooth with HAPt from cattle bone 100 mg/L(w/v); Magnification (500X)

4. Conclusions

The use of 100 mg/L of HAPt derived from cattle foreleg bone (*Os metacarpus*) reduced tooth mass loss when teeth were submerged in a pH-5, 1M concentration of acetic acid buffer solution. Moreover, HAPt protected the teeth from demineralization and cracking while submerged for up to 48 h.

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6. Authors' Contributions

Muhammad Irfan Said was fully responsible for the process of data collection, data analysis, interpretation, compilation, correspondence and publication of the articles; Farida Nur Yuliati played a role in providing samples and writing the articles; Asdar Gani played a role in data collection, data analysis and processing, and writing the article; Paulina Taba played a role in statistical data processing and data interpretation, and writing and improving sentences in the articles.

7. Conflicts of Interest

The authors declare no conflict of interest.

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