Effect of Different Formulations and Application Methods of Coral Calcium on its Remineralization Ability on Carious Enamel

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Abstract

BACKGROUND: Coral calcium is a new biomimetic product and dietary supplement which consists mainly of alkaline calcium carbonate.

AIM: The aim of the current study is to compare the remineralization effect of coral calcium in different formulations and application methods.

METHODS: A total of 35 extracted molars was collected, examined, and sectioned to obtain 70 sound enamel discs, all specimens were examined for calcium mineral content using energy dispersive analysis of X-rays (EDAX) coupled with scanning electron microscope. Hydroxyapatite (HA) nanoparticles were synthesized through wet chemical precipitation approach and characterized by transmission electron microscopy (TEM) and Fourier transform infrared (FT-IR) analysis. Teeth specimens were subjected to demineralization, and mineral content was measured, specimens were divided into ten groups according to the remineralizing agent used, where Groups 1–3 used 10, 20, and 30 weight % (wt.%) coral calcium gel, respectively, Groups 4–6 used 10, 20, and 30 wt.% coral calcium and nanohydroxyapatite mix gel, and Groups 7–9 used 10, 20, and 30 wt.% coral calcium and alkaline calcium carbonate. All groups showed a statistically significant decrease in calcium level after demineralization, all groups showed a statistically significant increase in calcium content after remineralization except for the control group. Moreover, Groups 2 and 8 showed the highest increase in calcium level after remineralization.

RESULTS: The TEM and FT-IR analysis confirmed the formation of rod shape HA in nanoparticles size range. Coral calcium showed a significant remineralizing effect on carious enamel (demineralization) with an optimum concentration of 20 wt. %.

CONCLUSION: Coral calcium showed a significant remineralizing effect on carious enamel (demineralization) with an optimum concentration of 20 wt. %.

Introduction

Dental caries is one of the most widespread diseases which lead to the destruction of tooth structure by acid production by oral cariogenic pathogens due to carbohydrate fermentation [1], [2], [3]. The natural remineralization procedure of enamel depends on the salivary calcium and phosphate ions in addition to fluoride [4], [5]. The strategy of dental caries in demineralization is performed through secretion of acid by bacteria in dental plaque biofilms [6]. Dental caries is a cyclic disease composed of alternating cycles of de- and re-mineralization. The process of de- and re-mineralization is controlled by the degree of acid attack versus the saturation of saliva with apatite minerals [7]. Providing the proper change in conditions, re-mineralization will dominate, resulting in lesion repair. To stimulate lesion re-mineralization, elevated calcium and phosphate concentration in contact to the de-mineralized lesion will enhance the process [8].

Dental enamel is composed of approximately 96 weight % of minerals, similar to hydroxyapatite (HA) Ca_{10}(PO_4)_{6}(OH)_2. The structural nature of apatite crystal means that they allow many substitutions in its crystal lattice without changing identity. In apatite crystals, the substitution of calcium ions may occur by ions such as sodium, zinc, and strontium, while phosphate in the lattice can be substituted by carbonate. The process of interaction between enamel and oral fluids is ruled by the dissolution behavior of the apatite mineral, of which enamel is composed [9], [10].

HA nanoparticles are recently considered as an enamel substitute due to chemical similarity. Many dental studies recommend to remineralize enamel using HA or amorphous calcium phosphate (ACP) [11], [12]. Calcium-based compounds could be efficiently used for healing of biological dental tissues [13], [14], [15], [16]. Despite the complicated hierarchical structure of enamel, it is now clear that the main structure of enamel is generally consists of 20–40 nm HA nanoparticles. HA particles in nanosize could serve as an analog compound that mimics the normal biological apatite
Preparation and characterization of HA nanoparticles

HA nanoparticles were prepared through the wet chemical process [15], [22]. The precursors use calcium nitrate \([\text{Ca(NO}_3\text{)}_2]\) and ammonium phosphate \([\text{(NH}_4\text{)}_2\text{PO}_4]\). NH_4OH was used as a precipitating agent. First, an aqueous solution of Ca(NO_3)_2 (0.042 M) was freshly prepared and put in a 500 ml three-neck flask. Another two separate solutions in two different flasks of (NH_4)_2PO_4 (0.025 M) and NH_4OH were also prepared. The last two solutions were added dropwise through to the Ca(NO_3)_2 solution while the pH was monitored at 10. Through the addition process, a white precipitate was formed that taken as an indication for the formation of the HA nanoparticles. Finally, all the HA powders obtained were sintered at 300°C. The HA with Ca/P ratio equal 1.67 was prepared according to the following equation:

\[
10\text{Ca(NO}_3\text{)}_2 + 4\text{H}_2\text{O} + 6\text{(NH}_4\text{)}_2\text{HPO}_4 + 8\text{NH}_4\text{OH} = \text{Ca}_{10}(\text{PO}_4)\text{6(OH)}_2 + 20\text{NH}_4\text{NO}_3 + 46\text{H}_2\text{O}
\]

Remineralization analysis

Initial mineral content examination for calcium mineral content was measured before any treatment via scanning electron microscope coupled with energy dispersive analysis of X-rays (EDAX). The procedure of demineralization (artificial caries) was induced by individual immersion of the specimens in 10 ml of a demineralizing solution of pH 4.3 at 37°C for 14 days where the solution was changed every other day. The solution contained: 0.1 mol/l lactic acid buffer, 0.2% polyacrylic acid (Carbopol® 980 - DEG Importadora de Produtos Químicos Ltda., São Paulo, SP, Brazil), and 0.03 ppm F (Gen-phos HA - Hôpitalia Cirúrgica Catarinense Ltda., Florianópolis Santa Catarina, Brazil) at pH 5.0. To avoid fungal growth in the demineralization solution, 0.18% methylparaben was added to it. Calcium mineral content was measured after induction of demineralization (artificial caries) using EDAX.

Remineralizing gel was prepared by the addition of different formulation of coral calcium (cc) to cellulose gel purchased from Sigma Aldrich. The specimen was divided equally into ten groups, where Groups 1–3 used 10, 20, and 30 weight % (wt.%) coral calcium gel, respectively; Groups 4–6 used 10, 20, and 30 wt.% coral calcium and nanohydroxyapatite mix gel; Groups 7–9 used 10, 20, and 30 wt.% coral calcium with argon laser activation (5 s); and Group 10 (control group) without using a remineralizing agent. The final examination of the calcium mineral content was re-measured after the remineralization. The calcium levels were calculated.
mean values were obtained, and percentage of change was calculated to detect the remineralization ability of each gel. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests and showed parametric (normal) distribution. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples.

Results

The TEM image of the prepared HA nanoparticles was represented in Figure 1. It was observed that HA particles were created as rod shape flakes with an average size (80–100 nm length) and (15–20 nm width).

Table 1: Mean and SD of the percentage of calcium remineralization (after application of remineralizing gel) of the different groups

| Groups                   | Percentage of calcium remineralization | SD  |
|--------------------------|----------------------------------------|-----|
| Mean                     |                                        |     |
| Group 1 (10% cc)         | 38.62b                                 | 6.00|
| Group 2 (20% cc)         | 48.64c                                 | 5.95|
| Group 3 (30% cc)         | 39.06b                                 | 6.19|
| Group 4 (10% cc+HA mix)  | 39.06b                                 | 7.09|
| Group 5 (20% cc+HA mix)  | 33.04b                                 | 8.27|
| Group 6 (30% cc+HA mix)  | 37.29b                                 | 6.23|
| Group 7 (10% cc+argon laser) | 35.38b                               | 6.39|
| Group 8 (20% cc+argon laser) | 47.49c                               | 5.14|
| Group 9 (30% cc+argon laser) | 36.20b                               | 4.45|
| Group 10 (control)       | 7.26a                                  | 3.67|

*p-value <0.001*

Mean values having dissimilar letters denote statistical significance differences *significant-set at (p<0.05).

In addition, a statistically significant difference was found between (Group 8) and each of (Group 1), (Group 3), (Group 4), (Group 5), (Group 6), (Group 7), and (Group 9) where (p < 0.001).

Discussion

The HAnanoparticles were synthesized through wet chemical precipitation approach. This technique is characterized by being simple, using inexpensive raw materials, and low reaction temperature. The synthesized HA was characterized using TEM and FT-IR analysis. The TEM image observes the morphologies of the particles and confirms that the particles fall into the nano-sized range. The FT-IR spectra confirmed the
The enamel crystals start a demineralization process when the bacterial acids drop the pH below 5.5 (critical pH). A white spot lesion is detected in early phases as a result of loss of calcium and phosphate from surface and subsurface. The demineralization of crystals can be reversed on the condition that the acidic pH of the biofilm is buffered. Normal healthy saliva demonstrates a major role in helping to regain a remineralization favorable pH at the tooth surface by its buffering capacity due to its slight alkalinity and enriched mineral content [4].

The process of remineralization requires removal of the dietary carbohydrate and subsequent elevation in the pH of the biofilm to 7.0. When the pH is above the critical level (alkaline), demineralization will be reduced, the mineral begins to regain calcium and phosphate from saliva, and from other topical sources such as mouthwashes, toothpastes and dietary components, diffuses into the tooth and, with the help of remineralizing agents, builds on the surface. Newly formed crystal is composed of a layer of well-established minerals, together with deficient minerals. The crystal on the surface is less soluble to the attack of acid than the original HA crystals [23].

Coral calcium is calcium carbonate natural material with many other minerals which are essential for the human body; it increases the alkalinity of the enamel surface as well as enhancing remineralization of initial enamel lesion [24].

Therefore, in this study, coral calcium was tested as remineralizing agent in different concentrations and forms, whereby, it was used in 10%, 20%, and 30 wt.% concentrations using argon laser to increase the temperature so to enhance the precipitation of calcium into the enamel surface and in a combined form with nanohydroxyapatite to promote its remineralization process. The specimens were evaluated using EDXA to determine the amount of calcium after placing them in demineralizing solution and after remineralization.

The process of enamel demineralization depends on pH, calcium, phosphate, and fluoride contents of teeth, these factors determine the level of mineral saturation. Thus, a sub-saturation condition can result in dissolution of HA crystals into the tooth and diffusion of calcium ions towards the enamel surface. A hyper-saturation of these ions on the enamel surface can lead to re-deposition of HA crystals known as remineralization, and consequently to the formation of an intact superficial layer on enamel surface [25].

Conclusion

Coral calcium in all its forms showed the potential to remineralize initial enamel caries lesions. A suspension of 20 wt.% coral calcium had the optimal concentration for remineralizing early enamel caries. Within the restrictions of the current study, we can conclude that coral calcium of optimal concentration
could be beneficial in enhancing remineralization with regular usage.

**Recommendation for future works**

It is recommended to perform in vivo study about the remineralization effects of the coral calcium over a long period to get a more accurate result as it is a promising natural material.

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**References**

1. Farooq I, Ali S, Siddiqui IA, Al-Khalifa KS, Al-Hariri M. Influence of thyroquinone exposure on the micro-hardness of dental enamel: An in vitro study. Eur J Dent. 2019;13(3):318-22. PMid:31618784
2. Ali S, Farooq I. A review of the role of amelogenin protein in enamel formation and novel experimental techniques to study its function. Protein Pept Lett. 2019;26(12):880-6. PMid:31364509
3. Alfaroukh R, Elembaby A, Almas K, Ali S, Farooq I, Bahammam M, et al. Oral Biofilm formation and retention on commonly used dental materials: An update. Odonto-Stomatol Rev. 2020;37(6):510-7. PMid:32201577
4. Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: A review. F1000Res. 2020;9:171. PMid:32057374
5. Ali S, Farooq I, Al-Thobity AM, Al-Khalifa KS, Alhooshani K, Sauro S. An in-vitro evaluation of fluoride content and enamel remineralization potential of two toothpastes containing different bioactive glasses. Biomed Mater Eng. 2020;30(5-6):487-96. PMid:31594192
6. Margolis HC, Moreno EC. Kinetics of hydroxyapatite dissolution in acetic, lactic, and phosphoric acid solutions. Calcif Tissue Int. 1992;50(2):137-43. PMid:13159080
7. Pearce EIF, Moore AJ. Remineralization of softened bovine enamel following treatment of overlying plaque with a mineral-enriching solution. J Dent Res. 1985;64(3):416-21. PMid:3858591
8. Kielbassa AM, Muller J, Germhardt CR. Closing the gap between oral hygiene and minimally invasive dentistry: A review of the resin infiltration technique of incipient (proximal) enamel lesions. Br Dent J. 2009;207(9):425.
9. Wang J, Layrolle P, Stigter M, De Groot K. Biomimetic and electrolytic calcium phosphate coatings on titanium alloy: Physicochemical characteristics and cell attachment. Biomaterials. 2004;25(4):583-92. PMid:14607496
10. Okazaki M, Takahashi J, Kimura H. Crystallinity patterns of fluoridated hydroxyapatites before and after incubation in acidic buffer solution. Caries Res. 1984;18(6):499-504. PMid:6593121
11. Featherstone JD. Remineralization, the natural caries repair process—the need for new approaches. Adv Dent Res. 2009;21:4-7.
12. Vandiver J, Dean D, Patel N, Bonfield W, Ortiz C. Nanoscale variation in surface charge of synthetic hydroxyapatite detected by chemically and spatially specific high-resolution force spectroscopy. Biomaterials. 2005;26(3):271-83. PMid:15262469
13. Zaki DY, Zaazou MH, Khalilaf ME, Hamdy TM. In vivo comparative evaluation of periapical healing in response to a calcium silicate and calcium hydroxide based endodontic sealers. Open Access Maced J Med Sci. 2019;6(8):1-5. PMid:30159080
14. Hamdy TM, Mousa SM, Sherief MA. Effect of incorporation of lanthanum and cerium-doped hydroxyapatite on acrylic bone cement produced from phosphogypsum waste. Egypt J Chem. 2019;63:22-23.
15. Hamdy TM, Sanjour SH, Sherief MA, Zaki DY. Effect of incorporation of 20 wt% amorphous nano-hydroxyapatite fillers in poly methyl methacrylate composite on the compressive strength. Res J Pharm Biol Chem Sci. 2015;6(3):975-8585.
16. Hamdy TM, El-Korashy SA. Novel bioactive zinc phosphate dental cement with low irritation and enhanced microhardness. e-J Surf Sci Nanotechnol. 2018;16:431-5.
17. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomed Mater. 2009;4(3):034104. PMid:19498220
18. Kim MY, Kwon HK, Choi CH, Kim BI. Combined effects of nano-hydroxyapatite and NaF on remineralization of early caries lesion. Key Eng Mater. 2007;330-332:1347-50.
19. Hamdy TM. Polymers and ceramics biomaterials in orthopedics and dentistry: A review. e-J Surf Sci Nanotechnol. 2018;63:22-23.
20. Orsini G, Proccacciini M, Manzoli L, Giuliani F, Lorenzini A, Putignano A. A double-blind randomized-controlled trial comparing the desensitizing efficacy of a new dentifrice containing carbonate/hydroxyapatite nanocrystals and a sodium fluoride/potassium nitrate dentifrice. J Clin Periodontol. 2010;37(6):510-7. PMid:20507374
21. Yamagishi K, Onuma K, Suzuki T, Okada F, Tagami J, Otsumi M, et al. Materials chemistry: A synthetic enamel for rapid tooth repair. Nature. 2005;433(7028):819. PMid:15729330
22. Rodríguez-Lugo V, Karrhik TV, Mendoza-Anaya D, Rubio-Rosas E, Cerón LS, Reyes-Valderrama MI, et al. Wet chemical synthesis of nanocrystalline hydroxyapatite flakes: Effect of pH and sintering temperature on structural and morphological properties. R Soc Open Sci. 2018;5(8):180962. PMid:30225084
23. Jones EM, Cochrane CA, Percival SL. The effect of pH on the extracellular matrix and biofilms. Adv Wound Care. 2015;4(7):431-9. PMid:26155386
24. Brockmann D, Janse M. Calcium and carbonate in closed marine aquarium systems. In: Advances in Coral Husbandry in Public Aquariums. Netherlands: Burgers’ Zoo; 2008. p. 133-42. Available from: https://www.researchgate.net/publication/228361536_Calcium_and_carbonate_in_closed_
25. Pretty IA, Ingram GS, Agalamanyi EA, Edgar WM, Higham SM. The use of fluorescein-enhanced quantitative light-induced fluorescence to monitor de and re-mineralization of in vitro root caries. J Oral Rehabil. 2003;30(12):1151-6. PMID:14641655

26. Kumar VM, Govind GK, Siva B, Marish P, Ashwin S, Kiran M. Corals as bone substitutes. J Int Oral Health. 2016;8(1):96-102.

27. Ali Abdelnabi NK, Othman MS. Evaluation of re-mineralization of initial enamel lesions using nanohydroxyapatite and coral calcium with different concentrations. Egypt Dent J. 2019;65(2):3713-8. PMid:20619311

28. Huang S, Gao S, Cheng L, Yu H. Combined effects of nano-hydroxyapatite and Galla chinensis on remineralisation of initial enamel lesion in vitro. J Dent. 2010;38(10):811-9. PMid:20619311

29. Sonali Sharma LC, N. Hegde M, Sadananda V, Matthews B. Evaluation of efficacy of different surface treatment protocols by laser fluorescence: An in vitro study. Dent Oral Craniofacial Res. 2017;3(4):1-5.