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Synthesis of Calcium Phosphate Composite Organogels by Using Emulsion Method for Dentine Occlusion Materials

C Nopteeranupharp[1], K Akkarachaneeyakorn[1], A Songsasaen[1]

[1]Faculty of science, department of chemistry, Kasetsart University, 50 Ngamwongwan street, Lat Yao, Chatuchak, Bangkok, Thailand 10900

Abstract. Dentinal hypersensitivity (DH) is one of the most human's problems caused by the erosion of enamel. There are many methods and materials to solve this problem. Calcium phosphate is an excellent alternative for curing this symptom because of its osteoconductivity, and biocompatibility properties. The low-cost and low-toxicity calcium phosphate nanogel was fabricated by using emulsion method and characterized by using TEM, EDX, and DLS techniques. The results showed that P123 (poly (ethylene oxide)$_{19}$-*block*-Poly (propylene oxide)$_{69}$-*block*-poly (ethylene oxide)$_{19}$) has played a major role as template and gel formation, SDS was used as a surfactant to form water-in-oil emulsion nanodroplets with circle-like shape. Moreover, the ability of synthesised organogel to occlude the exposed dentine tubules was tested on the model of human's dentine slices. The results showed that calcium phosphate composite organogel can be efficiently occluded on dentine slice, characterized by SEM technique, after 1 day.

1. Introduction

Dentinal hypersensitivity (DH) is described as a "sharp pain of short duration which occurs when the stimulus of chemical or thermal origin comes in contact with an exposed dentine surface" [1]. It is mainly caused by the erosion of enamel due to human's habits in daily life such as eating and drinking. Within enamel, there are numerous tiny dentine tubules filled with fluid. When enamel is destroyed, dentine tubules can be easily contacted with the outer stimuli and the fluid in dentine tubules are oscillated [2].

From previous works, many researchers have been continually studying on how to reduce DH symptom and producing various desensitizing agents such as bioactive glass (BG), fluoride, oxalate, and hydroxyapatite (HA) [2]. Calcium phosphate (CaP), especially hydroxyapatite, is one of the most popular materials widely used in biomedical, biomaterial and bioengineering applications because of its biocompatibility, osteoconductivity, non-inflammable, bioactive, biodegradable and non-toxicity properties. Moreover, the methods and conditions of CaP preparation such as sol-gel, microwave, and hydrothermal methods are reported to have an effect on the sizes, morphologies and phases of CaP [3-5].

Herein, calcium phosphate composite organogels were synthesized by the main components of P123 (poly (ethylene oxide)$_{19}$-*block*-Poly (propylene oxide)$_{69}$-*block*-poly (ethylene oxide)$_{19}$), compared with calcium phosphate nanoparticle without the addition of P123 to study the gel formation and the effect of it on the emulsion formation, SDS (sodium dodecyl sulfate) as surfactant, eucalyptus oil as an organic phase, and calcium and phosphate source in aqueous phase using emulsion method. Emulsion methods are known to be low-cost procedures performing under mind condition and can be used to produce small particle sizes (in nanometer to submicron) [6]. Pluronic polymer, poly (ethylene oxide)$_{19}$-*block*-Poly (propylene oxide)$_{69}$-*block*-poly (ethylene oxide)$_{19}$ (P123), has widely used as a

template to form gel, and control the size, structure and morphology of particles [7]. In this study, P123 was added in order to form a gel which could facilitate the application of the product on the exposed teeth. Eucalyptol was used as an organic solvent due to its low toxicity. In addition, calcium phosphate nanogels were tested in vitro with human's dentine slices to demonstrate the occlusive performance of fabricated organogel.

2. Material and methods

All chemicals were of analytical grade without any further purification. Poly (ethylene oxide)$_{19}$-*block*-Poly (propylene oxide)$_{69}$-*block*-poly (ethylene oxide)$_{19}$ (P123) (M.W. 5800 Da), eucalyptol, sodium dodecyl sulfate (SDS), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma Aldrich.

2.1. Synthesis of calcium phosphate nanoparticles by using microemulsion method

Five milititres of eucalyptol was transferred in vial containing SDS (7.7×10^{-4} mol, 0.222 g) and then stirred vigorously overnight. Then, 100 µl of $CaCl_2 \cdot 2H_2O$ (7.4×10^{-4} mol, 0.1095 g) was added into the vial prior to be kept stirring vigorously for further 30 minutes. Two mililitres of Na_2HPO_4 aqueous solution (7.2×10^{-4} mol, 0.128 g) was added dropwise into the same vial under vigorous stir. The solution was stirred for another 30 minutes to complete the reaction before being left stand still for approximately 15 minutes to allow the phase separation. Finally, the oil fraction was collected and kept in another vial.

2.2. Synthesis of calcium phosphate composite organogel by using microemulsion method

0.2 g of P123 (M.W. 5800 Da, 3.5×10^{-5} mol) was mixed with 5 ml of eucalyptol and kept stirring overnight. SDS (7.7×10^{-4} mol, 0.222 g) was added into the vial before being stirred vigorously overnight once again. Then, 100 µl of $CaCl_2 \cdot 2H_2O$ (7.4×10^{-4} mol, 0.1095 g) was added into the vial and then kept stirring vigorously for another 30 minutes. Na_2HPO_4 aqueous solution (7.2×10^{-4} mol, 0.128 g, 2 ml) was added dropwise into the vial under vigorous stir. The solution was stirred for 30 minutes before being aged at room temperature for phase separation (approximately 15 minutes) and the oil fraction was collected and evaporated under vacuum at room temperature to remove solvent.

2.3. Characterization

The morphology and size of synthesized calcium phosphate nanoparticles and the synthesized calcium phosphate composite organogel were characterized by using transmission electron microscope (TEM). Briefly, 20 µl of samples was dispersed into 100 µl of ethanol and then sonicated for 10 minutes before dropped onto carbon-coated copper grids and left to dry in air overnight. Dynamic light scattering (DLS) was used to confirm the size of nanoparticles contained in the emulsion and organogel from TEM. Shortly, 3 ml of samples was added into the cuvette for analysis using Malvern Zetesizer instrument.

2.4. Dentine tubule occlusion and mineralization

The model of dentine tooth slices were kindly supported by the faculty of dentistry, Mahidol University and prepared as previous description [8]. They were kept in 1:1 ethanol: water to prevent the growth of some bacteria. Firstly, each of dentine slices was sonicated in deionized water for 15 minutes and dried in air. Then, the samples of calcium phosphate composite organogel were applied on dentine slices and kept under moisture condition for 5 days. After that, the dentine slices were washed by ethanol extensively in order to remove the excess of gel and oil. Finally, the dentine slice samples were placed on carbon stub and sputter-coated with gold ready to be characterized by scanning electron microscope (SEM) for investigating the occlusion efficiency of samples on tooth.

3. Results and discussions

Calcium phosphate composite organogel was synthesized by emulsion method in the presence of P123, SDS, eucalyptol, $CaCl_2$ and Na_2HPO_4 as precursors. In the beginning, colorless solution was occurred when P123 was added into eucalyptol (figure 1A). The solution then became turbid after the

addition of surfactant, SDS (figure 1B). Calcium and phosphate source in aqueous solution were respectively added into the solution to form the emulsion. Finally, the aqueous phase (lower) and oil phase (upper) were separated (figure 1C), and then the water-in-oil emulsion layer was collected into the new vial (figure 1D). Compared with the calcium phosphate nanoparticles without P123 by emulsion method, the calcium phosphate composite organogel was more obviously viscous. The results confirmed that P123 was performed a major role in gel formation.

A **B** **C** **D**

Figure 1. Digital photograph showing process of calcium phosphate composite organogel synthesis after the addition of (A) P123 (B) SDS (C) calcium and phosphate aqueous solution and (D) the oil fraction containing calcium phosphate nanocomposite.

The size and morphology of calcium phosphate nanoparticles contained in organogel compared to calcium phosphate nanoparticles prepared in the absence of P123 were characterized by TEM. The image of calcium phosphate nanoparticles (figure 2-left) showed the circle-like shape with approximately 10-60 nm diameters and connected with fibocous structure with 5 nm thickness. Whereas, the TEM image of calcium phosphate nanoparticles contained in organogel (figure 2-middle) showed the circle-like shape with 10-40 nm diameters and the network of calcium phosphate with 5 nm thickness. Moreover, the large particles, approximately 90-110 nm diameters, which presumably from agglomeration of the small particles by themselves were observed. Referred from previous paper [7], the results showed that SDS was the main component to form water-in-oil emulsions and nanofibrous structure, whereas the addition of P123 as template could still stabilize the spherical structure. However, nanofibrous structure might be changed to the thicker network nanofibers. EDX technique was used to characterize the components in calcium phosphate composite organogel. The EDX spectra (figure 2-right) showed the peak of carbon, phosphorous, calcium, oxygen, and sulfur which confirmed that calcium phosphate nanoparticles could be synthesized by emulsion method. To confirm the sizes of calcium phosphate nanoparticles and organogel, DLS technique was used and the result indicated that the major size of calcium phosphate nanoparticles was 80 nm approximately (figure 3), which is relevant in nanometer range, but calcium phosphate organogel could not be measured by DLS technique because the structure of organogel was not indiscrete circle-shape structure.

The ability of synthesized calcium phosphate composite organogel to occlude dentine tubules was tested in vitro with human's dentine slices. The synthesized organogel was applied on the dentine slices, which kept into the humid condition for 1 and 5 days. Dentine slices were washed with ethanol to eliminate the excess of SDS, P123 and other matrixes before being characterized by SEM. Compared to the dentine slice without the application of calcium phosphate organogel (figure 4A), the results showed that the dentine tubules were almost fully occluded after the calcium phosphate after 1 day of organogel application (figure 4B) and almost covered all the surface of dentine slice after 5 days (figure 4C). As the results, it confirmed that the mineralization process on dentine with high stability was occurred. Due to the fact that dentine mainly consists of hydroxyapatite (HAp), it can lead to the biomineralization of synthesized calcium phosphate nanoparticles on the dentine tubules.

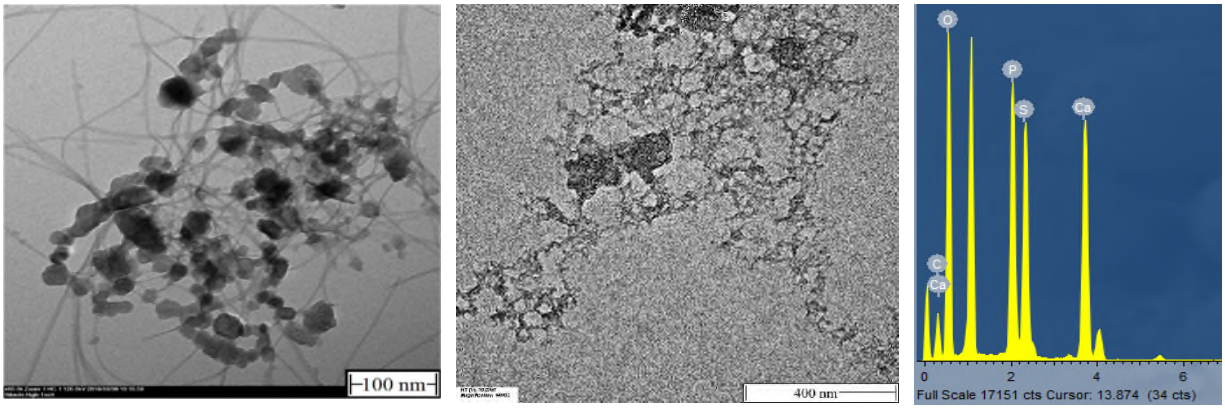

Figure 2. TEM images of calcium phosphate nanoparticles without P123 (left), calcium phosphate with P123 (middle), and EDX spectra of calcium phosphate from TEM (right).

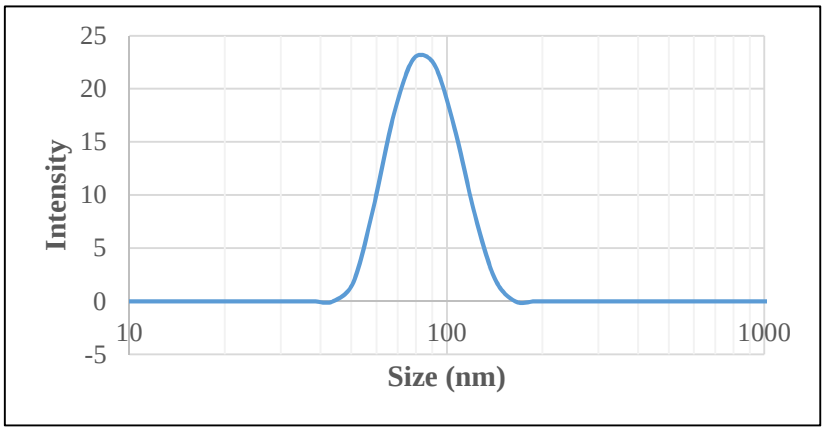

Figure 3. DLS peak of calcium phosphate nanoparticles

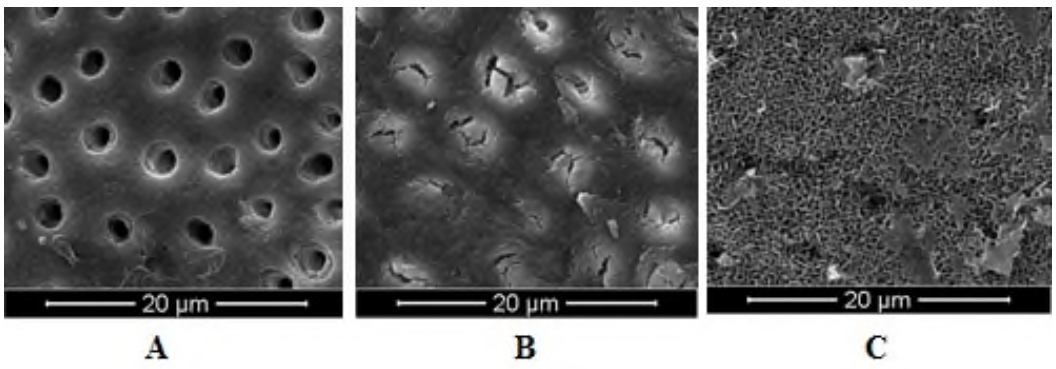

Figure 4. SEM images of dentine slices washed with ethanol (A) before applying calcium phosphate organogel and after applying calcium phosphate organogel for (B) 1 day and (C) 5 days, showing the occlusion of the dentine tubules with synthesized calcium phosphate.

4. Conclusion

In conclusion, calcium phosphate composite organogel can be synthesized by using emulsion method, which used eucalyptol as low-toxicity organic phase, P123 as a template for calcium phosphate and gel formation, and SDS as surfactant to form water-in-oil emulsion. This method is easy to synthesize, low-cost, and require mild condition. TEM and DLS techniques showed that size and morphology of organogel was circle-like in nanosize. Moreover, EDX spectra confirmed the component of calcium

and phosphate in organogel. For the dentine occlusion efficiency, calcium phosphate organogel was efficiently occluded the dentine tubules in vitro within 1 day. In the future, organogel will be improved for finding the optimum condition to synthesize in order to apply for dentine occlusion.

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