Synthesis of calcium phosphate composite organogels by using castor oil and sorbitan monopalmitate based for dentine occlusion material

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Abstract. Calcium phosphate composite organogel was synthesized from a mixture of sorbitan monopalmitate as emulsifier and castor oil as oil phase at 60 °C to establish emulsion system with calcium chloride and di-sodium hydrogen phosphate aqueous solution as water phase. The heated mixture was cooled at room temperature to form organogel. The prepared organogels were investigated by thermogravimetric analysis (TGA), scanning electron microscope (SEM), transmission electron microscope (TEM) and cytotoxicity test. The prepared organogels of 4:3 and 1:1 in oil/water content ratio were white and gritty in nature, which resulted in calcium phosphate nanoparticles with spherical shape of 1.3 to 1.9 μm and 1.2 to 2.3 μm, respectively. They were able to successfully occlude into dentine tubules within 1 day. In addition, the prepared organogels were found to have acceptable toxicity level for daily human use with high concentration up to 1600 μg/ml. Hence, these organogels showed the promising results to be developed as an effective dentine occlusion material in relieving of dentin hypersensitivity.

1. Introduction
Tooth sensitivity also known as dentin hypersensitivity is a prevalent problem of adult. According to previous researches, one in seven patients (or 8 to 57%) presented in a dental clinic to treat the pain from sensitive teeth [1]. Hypersensitivity symptom is typically transient sharp pain caused by thermal, chemical, evaporative, tactile, osmotic, or mechanical stimuli on exposed dentinal tubules [2]. Exposure of the dentine to cause the hypersensitivity occurs from several factors, for example a removal of tooth enamel occurred by the use of a hard toothbrush, gum recession, tooth erosion resulted from consuming acidic foods, an effect of gastroesophageal reflux disease which then leaves the dentine tubule to expose, or their combinations [3]. To reduce tooth sensitivity, in this research a desensitizing product will be developed on the basis of dentine tubule occlusion by using bioactive organogel. Since hydroxyapatite (HAp, Ca₁₀(PO₄)₆(OH)₂) is not only the major component of bone, dental enamel and dentine [4], but also the most stable crystalline form of calcium phosphate under human body conditions as pH, temperature and composition of body fluids, the prepared organogel was then embedded with hydroxyapatite [5,6].

Castor oil is an unsaturated fatty acid, containing ricinoleic acid (12-hydroxy-cis-octadec-9-enoic acid) that can be extracted from seed of Ricinus communis. It is popular in various industries, such as pharmaceutical products, cosmetics and synthetic detergents, owing to its anti-inflammatory, antioxidant and anti-microbial activities [7]. Sorbitan monopalmitate (SMP) is a non-ionic surfactant,
containing palmitic acid and sorbitol in the chemical structure. As SMP can facilitate the formation of emulsion, it has been widely employed in various pharmaceutical and cosmetic products [8]. In this study, the synthesis of calcium phosphate composite organogel was performed by using the mixture of sorbitan monopalmitate and castor oil based with modified process from previous report [8]. Different ratios of oil/water were varied to study the effect of formation of organogels and structure of synthesized calcium phosphate nanoparticles. The prepared calcium phosphate composite organogels were characterized and investigated for their toxicities and abilities to occlude the dentine tubule on human dentin slices.

2. Experimental section

2.1 Materials
In synthesis of calcium phosphate composite organogel, sorbitan monopalmitate (SMP) (MW 402.57 g/mol) and castor oil were obtained from Sigma-Aldrich (Saint Louis, USA). Calcium chloride monohydrate (CaCl₂·H₂O) (MW 147.02 g/mol) and di-sodium hydrogen phosphate (MW 141.96 g/mol) were bought from Merck KGaA (Darmstadt, Germany). All reagents were used without further purification.

2.2 Preparation of calcium phosphate composite organogels
Organogels were prepared according to previous report [8] with a slight modification. Initially, 800 μl of castor oil was heated at 60 °C and added 0.23 g of sorbitan monopalmitate with stirring at 500 rpm for dispersion as the hot solution was transparent. A 100 μl of calcium chloride solution (7.4 × 10⁻⁴ moles) was dripped into the solution and stirred for 30 min at 700 rpm. An aqueous solution of di-sodium hydrogen phosphate solution (7.2 × 10⁻⁴ moles in 500 μl of water) was then added dropwise with stirring for 1 h. The hot solution was cooled down to ambient temperature to form gel 1 with [Ca]:[P] molar ratio of 1:1. For gel 2 and 3 the volume of water was subsequently varied from 500 to 700 and 800 μl however the [Ca]:[P] molar ratio of gels are maintained the same.

2.3 Characterization
The calcium phosphate composite organogels were heated from 25 to 800 °C at a rate of 10 °C/min to determine the inorganic content by TGA. TEM investigation was also performed by using a Hitachi HT7700 transmission electron microscope (Hitachi High Technologies, Hitachinaka-shi, Japan). For the morphology and sizes studies, organogels were dispersed in ethanol and then sonicated for 15 min. A 2 μl of the dispersion liquid were dropped onto carbon-coated copper grids and left in a desiccator overnight. The analysis was performed at an accelerating voltage of 120 kV. The surface morphology of calcium phosphate nanoparticles in organogels was investigated by using a FEI Quanta 450 scanning electron microscope (Field Electron and Ion Company (FEI), Hillsboro, Oregon, USA) with 20 kV accelerating voltage.

2.4 Cytotoxicity test
Organogels were tested for cytotoxicity on human dermal fibroblast cell line. Briefly, cells were treated with various concentrations of the organogel, which was previously sterilized by UV exposure, followed by an incubation at 37 °C in a 95% humidified incubator with 5% CO₂ for 24 h. To investigate the cytotoxicity in cell, the sulforhodamine B (SRB) test was conducted. SRB was dissolved from living cells using 10 mM Tris buffer to observe the optical density at 492 nm using a Biotek PowerWave XS micro-plate reader (Biotek, Winooski, Vermont, USA).

2.5 Dentinal tubule occlusion
Dentine slices were prepared for tubule exposure. Each organogel was applied onto the surface of dentine specimen, then left for 1 day under moist condition. After that, organogel was washed away with water before sonication for 15 min. All of dentine specimens were kept in a desiccator before morphological study under SEM with using 15 kV accelerating voltage.
3. Results and discussions
The synthesis of calcium phosphate composite organogels were performed with the mixture of SMP and castor oil at 60 °C to form gel. Three ratios of castor oil/water were examined to obtain different texture of gel 1, 2, and 3. All prepared gels were white and turbid. The gel 1 and 2 were non-homogenous and gritty in nature, while the gel 3 was more homogeneous, as it had the highest water content. The TGA profile for gel 1, 2 and 3 shows weight losses of water of 29.3, 31.2, and 46.6%, respectively, at below 100 °C (Figure 1). The temperature was much lower than that for similar material of 150 °C in the report of Wu et.al. [9]. The sharp change in the range of 280 to 420 °C was attributed to the decomposition of organic group, which typically occurs between 300 to 800 °C [9]. After the decomposition of gel 1, 2 and 3 at 800 °C the final weights were 6.6, 7.8 and 8.7%, respectively, which indicated inorganic products of calcium phosphate composites. The SRB assay was utilized for cytotoxicity test, according to the ISO 10993 parts 5 and 12, the prepared organogel shows the minor toxicity (toxicity grade 1, 75 to 99% of relative growth rate [10, 11]) at a high concentration of the organogel up to 1600 µg/ml (Figure 2).

![Figure 1. TGA curves of calcium phosphate composite organogels.](image1)

![Figure 2. Cytotoxicity test of calcium phosphate composite organogels.](image2)

The morphologies of calcium phosphate nanoparticles were investigated by TEM as shown in Figure 3. The nanoparticles presented spherical-like shape, which was very similar to the emulsion system investigated in the previous study [12]. The gel 1 contained particles of 1.3 to 1.9 µm diameter partly adhered with very small granules (Figure 3a). The gel 2 exhibited the nanoparticles with a dimension range of 1.2 to 2.3 µm (Figure 3b), while the gel 3 gave particles with an increased size of 2.1 to 3.6 µm (Figure 3c). Similarly, the morphology of prepared organogels confirmed by SEM images shows spherical calcium phosphate nanoparticles dispersing within the gel matrix (Figure 4).

To study ability as a bioactive filler material for dentine tubule occlusion the prepared calcium phosphate composite organogels were also examined on human dentin slice. According to SEM studies, gel 1 and 2 showed the efficiency to occlude the exposed dentinal tubules within 1 day by calcium phosphate nanoparticlest (Figure 5a and 5b). However, the tooth slice of gel 1 treatment exhibited partial tubule exposure from the incomplete occlusion. Owing to the size of dentine tubules with approximately of 1.7 - 2.4 µm (Figure 5c), the sizes of calcium phosphate nanoparticles in gel 3 were larger than the sizes of tubules. Therefore, the treatment of gel 3 was not be able to occlude tubules within 1 day.
4. Conclusion

In this work, the synthesis of calcium phosphate composite organogels for dentine tubule occlusion were successfully prepared via emulsion method using the mixture of SMP-castor oil base. The texture of organogels were either gritty or smooth white in nature depending on composition of the gel formulation. The calcium phosphate nanoparticles dispersed in the gel 1 and 2 matrix were spherical with the dimension range of 1.3 to 1.9 and 1.2 to 2.3 μm respectively. Both gels were able to plug into dentine tubules within 1 day. Moreover, the high concentration up to 1600 μg/ml of organogel was acceptable toxicity level for human. The results confirmed that the prepared calcium phosphate composite organogels can be effectively used as a bioactive filler material in the treatment of dentine hypersensitivity. However, the work should be developed continually for treatment in vitro with artificial saliva and acid test to examine the wear resistance and stability of materials. The transverse sectioning of filled dentine tubules should be perform to observe the penetration depth of deposited calcium phosphate. The different mole ratio of calcium/phosphate should be varied to improve particles size and structure prior to being used as the effective bioactive filler material.

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