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Synthesis of bone-like micro-porous calcium phosphate/*iota*-carrageenan composites by gel diffusion

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ABSTRACT

Brushite and octacalcium phosphate (OCP) crystals are well-known precursors of hydroxylapatite (HAp), the main mineral found in bone. In this report, we present a new method for biomimicking brushite and OCP using single and double diffusion techniques. Brushite and OCP crystals were grown in an *iota*-carrageenan gel. The aggregates were analyzed by scanning electron microscopy (SEM), X-ray diffraction (XRD), infrared spectroscopy (IR) and thermal gravimetric analysis (TGA). SEM revealed different morphologies of brushite crystals from highly porous aggregates to plate-shaped forms. OCP crystals grown in *iota*-carrageenan showed a porous spherical shape different from brushite growth forms. The XRD method demonstrated that the single-diffusion method favors the formation of monoclinic brushite. In contrast, the double diffusion method was found to promote the formation of the triclinic octacalcium phosphate OCP phase. By combining the different parameters for crystal growth in carrageenan, such as ion concentration, gel pH and gel density, it is possible to modify the morphology of composite crystals, change the phase of calcium phosphate and modulate the amount of carrageenan inclusion in crystals. This study suggests that *iota*-carrageenan is a high-molecular-weight polysaccharide that is potentially applicable for controlling calcium phosphate crystallization.

1. Introduction

Carrageenans are a family of highly flexible and water-soluble linear anionic polysaccharides consisting of repeated galactose units and anhydrogalactose. The ester sulfate and hydroxyl groups of the galactose units are responsible for their anionic polarity. There are three main commercial structures of carrageenan, including *kappa*, *iota* and *lambda*, which differ in the number and position of sulfate groups. These compounds are widely utilized in the food, pharmaceutical, cosmetics and textile industries. This wide use is due to their excellent functional properties, such as their thickening, gelling, suspending and stabilizing abilities [1,2].

Brushite ($CaHPO_4{\cdot}2H_2O$) and OCP ($Ca_8H_2(PO_4)_6{\cdot}5H_2O$) are the two main precursors of HAp ($Ca_{10}H_2(PO_4)_6(OH)_2$), which features layered structures [3–5]. Brushite crystals consist of $CaPO_4$ chains arranged parallel to each other with interlayered water molecules. OCP is composed of alternating apatite and hydrated layers. The distribution of calcium and phosphate ions in apatitic layers is similar to that of HAp, though they are less densely packed in hydrated layers due to existence of water molecules [6–8]. Recently, a great deal of attention has been focused on brushite and OCP crystals growth in gels as they are useful for explaining the mechanisms of apatitic biomineralization in calcified tissues and crystal deposition diseases. Gel model systems represent an interesting medium to induce template nucleation for crystals to understand in vivo mineralization in tissues [9–11]. In this regard, silica and gelatin are the most widely studied gels with respect to brushite and OCP crystallization [12–18]. Furuichi et al. recently reported a more quantitative study for brushite preparation in gelatin. These researchers produced monetite after dehydration of brushite in gelatin by gel growth [18].

Previous investigations of the crystal growth of carrageenan had focused on the effects of the gel in the case of asparagine monohydrate and calcium carbonates [19–21]. More recently, HAp nucleation and growth in *kappa*-carrageenan by the co-precipitation technique was demonstrated [22,23].

However, to the best of our knowledge, there is no study on synthesizing porous brushite and OCP crystals in carrageenan gels. In this study, we used *iota*-carrageenan for gel growth because it displays a greater hydrophilic character than *kappa*-carrageenan, due

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Table 1
Set of experiments used in this study.

Sample tube	Method	Concentration of *iota*-carrageenan in water (wt.%)	Molarity of $CaCl_2$	Molarity of Na_2HPO_4	Molar ratio of Ca/P	Type of crystal obtained
1	Single diffusion	1	0.19	0.12	1.58	Brushite
2	Single diffusion	2	0.19	0.12	1.58	Brushite + OCP
3	Double diffusion	2	1	0.8	1.25	Brushite + OCP
4	Double diffusion	2	1	0.2	5	OCP

to the additional sulfate groups presented in the galactose residue. A study of calcium phosphates crystallization in *iota*-carrageenan is thus important for identifying new gels for the in vitro biomineralization processes [24–26].

This study investigated the morphological properties of brushite and OCP crystals grown in *iota*-carrageenan.

2. Experimental

The procedure used to synthesize brushite and OCP crystals consisted of preparing *iota*-carrageenan (Sigma–Aldrich) gels and buffered solutions for several single- and double-diffusion experiments. For single tubes, *iota*-carrageenan was dissolved in deionized water and 7 cc of $CaCl_2$ solution was then added to the gel by adjusting the pH with HCl to maintain a value of 5.0. The tube of the diffusion cell was filled with the mixture and left at room temperature for 24 h until gelation occurred. Next, 7 cc of Na_2HPO_4 solution buffered with tris(hydroxymethyl)aminoethane/HCl at pH 7.4 was poured into the carrageenan cell, and diffusion took place at 25 °C for 3 weeks. In the case of the double diffusion method, U-tubes (vertical section: 20 cm, horizontal section: 12 cm, diameter: 2 cm) were filled with *iota*-carrageenan adjusted to pH 5. Buffered $CaCl_2$ and Na_2HPO_4 solutions at pH 7.4 were poured into both sides of the tubes separately. Finally, brushite and OCP particles were collected and washed several times with hot water to remove excess gel and then dried at 80 °C. Table 1 shows the set of experiments for the preparation of samples. Crystals in single tubes were collected from the upper part of the reaction vessel close to the interface between the gel and disodium hydrogen phosphate. For U-tubes, crystals were mostly formed in the bulk of the gel near the interface of the gel and disodium hydrogen phosphate. A schematic that indicates the positions where the samples were collected is shown in Fig. 1. The experiments were repeated for each set of samples in 10 tubes.

Chemical characterization of carrageenan and crystals was carried out using a Perkin-Elmer Spectrum One IR spectrometer integrated with an IBM personal computer. IR spectra were measured with four scans for each sample.

Surface morphology analyses of the crystals were carried out by means of a Hitachi scanning electron microscope S-3000N. The presence of calcium and phosphate on the crystal surface was determined by a Noran SIX NSS200 dispersive X-ray detector (EDX) attached to the SEM.

Powder X-ray diffraction patterns were collected at ambient temperature using a STOE StadiP diffractometer in transmission Debye–Scherrer geometry (monochromatic Cu $K\alpha_1$ radiation, wavelength $\lambda = 0.15406$ nm). Specimens were placed in 0.5 mm capillaries. The scattered intensity was detected by a one-dimensional curved position sensitive detector (linear PSD). The diffractometer goniometer radius was 130 mm.

The thermogravimetric analysis (TGA) was performed on a Mettler-Toledo TG/SDTA 851e. In each case, a 5 mg sample was examined under N_2 at a heating rate of 5 °C/min from 50 °C to 600 °C.

3. Results and discussion

3.1. Chemical characterization

The IR spectra of *iota*-carrageenan, analytical grade brushite (Sigma–Aldrich Company) as well as calcium phosphate crystals prepared in *iota*-carrageenan gel are shown in Figs. 2 and 3. The presence of sulfate esters in carrageenan (Fig. 2a) is well represented by three strong bands at 1263, 1384 and 580 cm^{-1}. The characteristic band appeared at 1027 cm^{-1} along with the peaks at 1072 and 1165 cm^{-1}, which are assigned to the C—C and C—O stretching vibrations of the pyranose ring in the solid form of carrageenan, respectively. The skeleton bending of pyranose ring

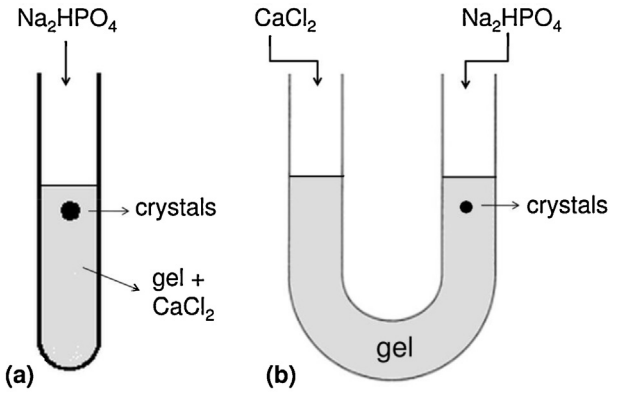

Fig. 1. Positions where crystals were collected from the tubes. (a) Single tubes were filled with *iota*-carrageenan and $CaCl_2$ mixture before adding Na_2HPO_4. (b) U-tubes were filled with *iota*-carrageenan and buffered $CaCl_2$ and Na_2HPO_4 solutions were poured in each side of the tubes separately.

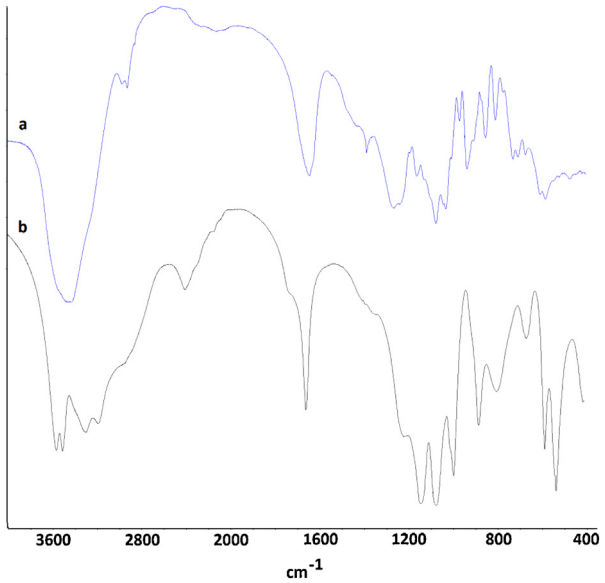

Fig. 2. The IR spectra of (a) *iota*-carrageenan and (b) analytical grade of brushite crystals supplied by Sigma–Aldrich Company.