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Carboxymethyl cellulose-*g*-poly (acrylic acid)/calcium phosphate composite as a multifunctional hydrogel material

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ABSTRACT

Multifunctional materials containing calcium phosphate to promote osteogenesis and capable to deliver drugs have gained increasing interest in the field of biomaterials. CMC-*g*-PAA hydrogel was evaluated to mineralize calcium phosphate during exposure to a solution of simulated body fluid. The formed composite was examined by scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA). The effect of mineralization process onto swelling behavior and drug release capability was evaluated at pH 2.1 and 7.4, similar to that of gastric and intestinal fluids respectively.

1. Introduction

Recently, new generation of bone-like composite materials with enhanced biocompatibility has been developed as a new alternatives for bone tissue regeneration purposes [1,2]. These hybrids which produced through calcium phosphate biomimetic mineralization are expected to initiate osteogenesis when implanted in bony sites [3–5].

Charged proteins presented in the extracellular matrix, decorated with acidic groups, are believed to promote the nucleation of hydroxyapatite [6]. Therefore, several research groups have focused on the design of charged hydrogels which mimic the proteins structure for the synthesis of bioinspired mineralized materials [7–13]. These materials are useful for regeneration of human bone although their chemical structure is different from the basic structure of natural bone. In addition, hydrogels have suitable properties to act as a multifunctional materials like high porosity, swellability in aqueous solutions [14] and capability to accommodate water-soluble compounds like drugs, therapeutically active proteins and peptides [15–17].

Using polysaccharides instead synthetic polymers as building blocks for preparing hydrogel have been developed for reducing the risks occurred due to the morbidity between human cells and synthetic polymers [15,18]. Acidic polymers such as polyaspartic acid, [19] polyacrylic acid, [20] alginate and phosphorylated alginate [21] have been used to mimic noncollagenous proteins for promotion the intrafibrillar mineralization. These polymers exhibited differences in their ability to control the mineralization process [22].

The current study aims to design hydrogel composed of CMC decorated with acidic functional groups and evaluating its capability to nucleate calcium phosphate under biomimetic mineralization process. Moreover, the study investigate the pH sensitivity of produced hydrogel composite as well as the release profile of bovine serum albumin, as a model for protein drug, at pH 2.1 and 7.4 similar to that of gastric and intestinal fluids respectively.

2. Experimental

2.1. Materials

CMC sodium salt (> 99.5%) with high viscosity was purchased from Fluka BioChemika. Comassie brilliant blue (G-250), bovine serum albumin (BSA), *N*, *N*′-methylenebisacrylamide (MBA) and acrylic acid (AA) was purchased from Sigma-Aldrich and used without further purification. Simulated body fluid chemicals were analytical grade and used as received without further purification.

2.2. Biomematic calcium phosphate mineralization of CMC-g-AA hydrogel

Graft copolymerization of acrylic acid onto polysaccharides using MBA as chemically crosslinker, was studied in details by

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different research groups [23]. In our work, 0.2 g CMC in 10 mL double distilled water was mixed with 1.7 mol/L of acrylic acid and 0.01 mol/L of MBA. After bubbling with N_2 gas for 20 min, 0.0015 mol/L ammonium persulfate as an initiator was added to the solution, and then the mixture was heated at 85 °C until complete gelation.

The *in-vitro* bioactivity of the CMC-*g*-PAA hydrogel disk samples (10 mm × 10 mm) were carried out by immersing in 2xSBF [24].

2.3. Swelling studies and In-vitro cumulative BSA release

The kinetic swelling of CMC-*g*-PAA hydrogel before and after mineralization was measured in two consecutive buffer solutions composed of phosphoric acid (0.054 mol), boric acid (0.040 mol) and acetic acid (0.042 mol) and adjusted to the required pH value by 0.2 N NaOH solution. Swelling% during 24 h was calculated by the following equation:

$$\text{Swelling\%} = 100[(W_t - W_0)]/W_0$$

where W_0 is the initial weight and W_t the final weight of the film at time *t*.

BSA-loaded CMC-*g*-PAA/calcium phosphate hybrids were prepared by incubation a cubic piece from CMC-*g*-PAA hydrogel in a glass vials with 10 mL BSA solution (25 mg/mL) for 3 days. After drying in vacuum oven, the hybrids were transferred to either a glass vials containing simulated gastric fluid buffer at pH 2.1 or simulated intestinal fluids at pH 7.4 and incubated at 37 °C without stirring for 12 h. After that, 1 mL of the release medium was taken periodically and assayed by Bradford method [25] at λ_{max} 595 nm.

2.4. Characterization

Scanning electron microscopy was carried out on Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K. V., magnification14 × up to 10,00,000. Both the CMC and CMC -*g*-PAA were characterized by FTIR (Mattson 5000 FTIR spectrometer) using KBr discs in the range of $4000-500\ cm^{-1}$. *X-ray diffraction* (XRD) patterns were recorded with an Empyrean Powder Diffractometer (CuK_α, 0.154 nm) between 5 and 70° 2θ with a step size of 0.01 deg/s. Samples were mounted on a silicon support. Thermogravimetric analysis was done on a PerkinElmer TGA7 thermogravimetric Analyzer under nitrogen.

3. Results and discussion

3.1. Hydrogel composite structure characterization

Graft copolymerization of acrylic acid onto CMC backbone was studied in detail (data not showed here) to optimize the swelling% of the hydrogel. Calcium phosphate nucleation preferentially enhanced by the graft co-polymer chains which have high density of carboxylic acid groups acting as Ca^{2+} binding sites [12]. The scanning electron microscopy (SEM) image of the prepared hydrogel in Fig. 1A exhibits a clear homogeneous 3D porous structures. After immersing in 2 × SBF for 7 days, the hydrogel surface revealed formation of calcium phosphate layer comprised of spherical with diameter mostly less than 2 μm as seen in Fig. 1B. These spherical particles connect together to form a cluster layers of amorphous calcium phosphate and/or hydroxyapatite [26].

The maps displayed in Fig. 2 exhibit fairly homogeneous elemental distributions on a few micrometers length scale which suggest that all materials are uniform over the micrometer length scale. Moreover, the EDX analysis detected the presence of Ca and P elements as the major constituents with Ca/P ratio 1.53, below the stoichiometric value of 1.67 for hydroxyapatite. Deviation from the stoichiometic value for hydroxyapatite are known in the literature and may be due to cationic or anionic substitutions [26]. In addition, this ratio may consider as an evidence for the presence of amorphous calcium phosphate as a precursor for hydroxyapatite.

The structural changes before and after biomimetic mineralization process was confirmed by FTIR spectroscopy as shown in Fig. 3A. Peaks at 1630, 2900 and 3439 cm^{-1} are related to carbonyl stretching of carboxylic group, asymmetric C–H stretching and O–H stretching vibration in CMC. However, CMC-*g*-PAA shows new

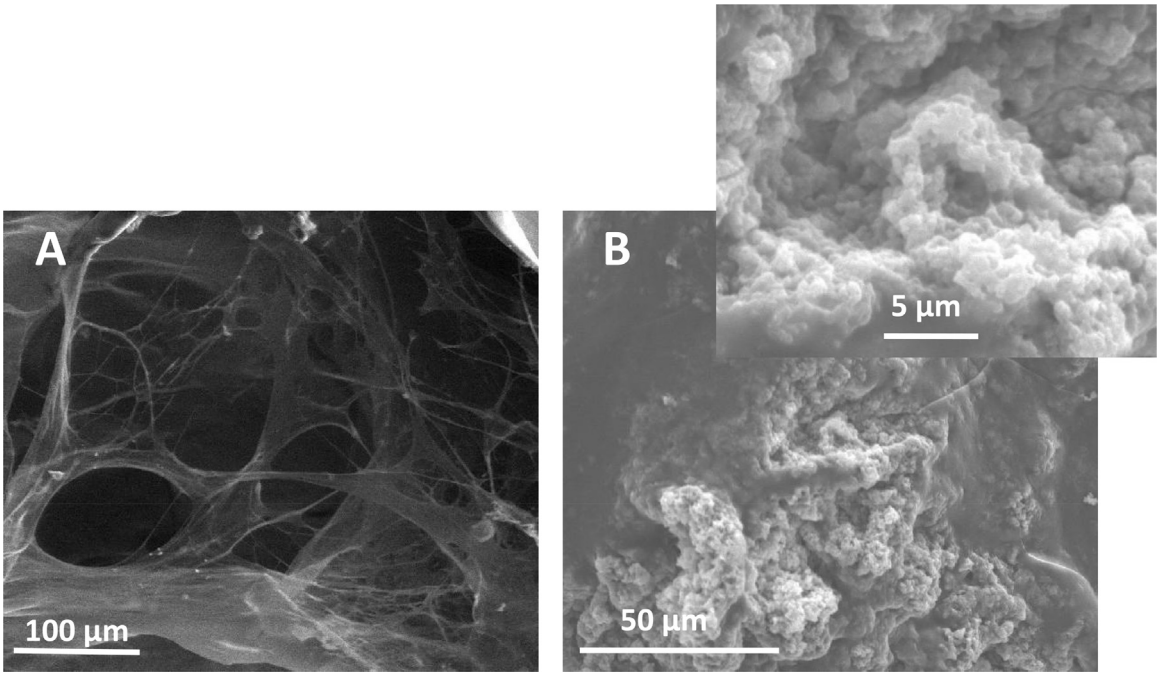

Fig. 1. SEM of CMC-*g*-PAA hydrogel before (A) and after (B) mineralization.