Colloids and Surfaces A: Physicochem. Eng. Aspects xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Calcium phosphate-polymer hybrid microparticles having functionalized surfaces prepared by a coaxially electrospray technique

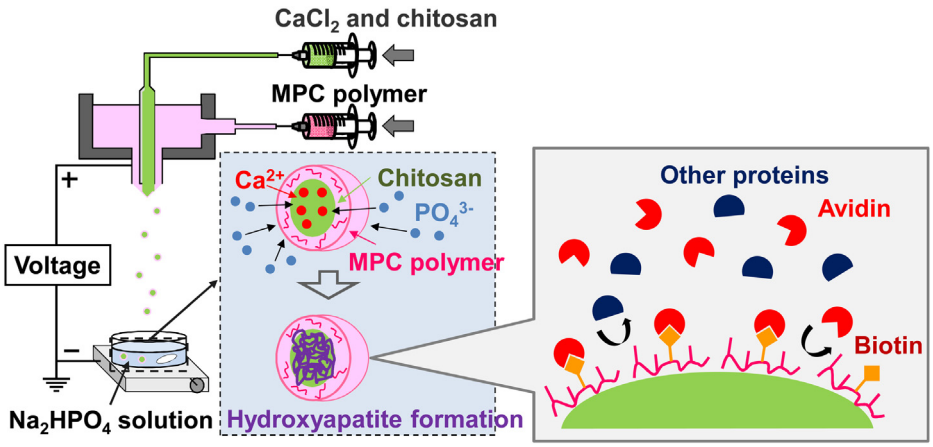
Tetsuya Matsuura, Tatsuo Maruyama*

Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan

HIGHLIGHTS

- Electrospraying prepared inorganic microparticles covered with biocompatible polymer.
- The microparticles did not adsorb proteins on their surfaces in a non-specific manner.
- Model substances were encapsulated within the microparticles with high efficiencies.
- Selective immobilization of a target protein on microparticle surfaces was achieved.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 11 October 2016
Accepted 14 October 2016
Available online xxx

Keywords:
Calcium phosphate
Electrospray
Inorganic microcapsules
Surface modification
Phosphoryl choline polymer

ABSTRACT

Microparticles composed of calcium phosphate (CaP) and chitosan covered with 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer were prepared in a single step by coaxial electrospraying. An aqueous solution containing calcium chloride, chitosan and an MPC polymer ethanol solution were electrosprayed from coaxial double needles into a phosphate solution. CaP/chitosan microparticles were successfully formed and their surfaces were simultaneously covered with MPC polymer. The resulting microparticles had an average diameter of around 400 μm. Investigation using fluorescently labeled MPC polymer revealed surface coverage of the CaP/chitosan microparticles with MPC polymer. The formation of CaP, mainly hydroxyapatite, was confirmed by X-ray diffraction measurement. A protein adsorption study revealed that bovine serum albumin (BSA) adsorption on the microparticles was effectively suppressed by the MPC polymer. Model substances (dextran and BSA) were successfully encapsulated within the microparticles, with high encapsulation efficiencies (more than 80%), in a single step by coaxial electrospraying. Finally, we succeeded in the selective immobilization of a target protein on the surface of the CaP/chitosan microparticles covered with MPC polymer.

1. Introduction

Calcium phosphate (CaP) is a major component of body tissue that has recently attracted increased attention as a biomaterial

* Corresponding author.
E-mail address: tmarutcm@crystal.kobe-u.ac.jp (T. Maruyama).

http://dx.doi.org/10.1016/j.colsurfa.2016.10.036
0927-7757/

because of its appropriate biodegradation and excellent biocompatibility. CaP is widely applied in artificial bone, drug delivery carriers, and cell scaffolds [1–5], and is also exploited in biomimetic mineralization research [6,7]. Although CaP crystals can be synthesized under mild conditions, it is usually difficult to control the crystal growth and the size (also shape) of CaP particles while preventing their aggregation [8]. To overcome these difficulties, there have been many reports on the preparation of CaP microparticles, including mechanochemical processing, chemical precipitation, microemulsion, and hydrothermal treatment [9–12]. An electrospray technique is an effective method to create a fast reaction to produce CaP microparticles in a single step. We reported the preparation of microparticles composed of calcium phosphate and organic polymer additives in a single step by electrospraying, and showed excellent encapsulation properties for various substances [13].

The surface properties of CaP particles play an important role in the application for life sciences, because its surface is in direct contact with biological substances. Indeed, CaP adsorbs a wide variety of molecules (proteins, organic acids, saccharides and nucleic acids) [14–18]. Therefore, the control of the surface properties of CaP particles is of great importance. To our knowledge, however, there has been no attempt to prepare CaP microparticles with low-fouling surfaces. In the present study, we used 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers for surface coating to prevent nonspecific protein adsorption on CaP microparticles. MPC polymers, which are inspired by the surface structure of biomembranes and synthesized by Ishihara et al., are well-known to show excellent suppression of protein adsorption onto various surfaces [19]. Here, we used a coaxial electrospray technique to prepare CaP-polymer hybrid microparticles covered with MPC polymer in a single step, and demonstrated low-fouling of the microparticles. Moreover, we succeeded in the selective immobilization of a target protein on the low-fouling surfaces of the microparticles using avidin-biotin interaction [20], which proposes high potential of CaP-polymer hybrid microparticles functionalized with biomolecules.

2. Materials and methods

Calcium chloride, chitosan (commercially available chitosan 10, with an aqueous solution viscosity below 20 mPa s when dissolved in 0.5 wt% acetic acid solution at 20 °C), acetic acid, disodium hydrogen-phosphate anhydride and ethanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). MPC polymer (Lipidure®-CM) and amine-modified MPC polymer (Lipidure®-NH01) were purchased from NOF America (White Plains, NY). Albumin–fluorescein isothiocyanate conjugate (FITC-BSA), fluorescein isothiocyanate-dextran (FITC-dextran, MW = 2,000,000 Da), albumin, tetramethylrhodamine isothiocyanate bovine (TRITC-BSA) and anti-mouse IgG (whole molecule)-TRITC antibody produced in goat (TRITC-IgG) were purchased from Sigma (St. Louis, MO). Tetramethylrhodamine isothiocyanate (TRITC) was purchased from Invitrogen (Carlsbad, CA). 9-(Biotinamido)-4,7-dioxanonanoic acid *N*-succinimidyl ester (Biotin-PEG_2-NHS) was purchased from Tokyo Chemical Industries (Tokyo, Japan). Avidin D, TRITC conjugate (TRITC-avidin) was purchased from Funakoshi (Tokyo, Japan). Other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

TRITC-labeled MPC polymer was prepared as follows. Amine-modified MPC polymer (87 mg) was dissolved in a triethylamine/acetate buffer (pH 8, 0.1 M, 9 mL). A dimethylformamide solution (1 mL) containing 30 mg of TRITC was added to the amine-modified MPC polymer solution. After 4 h at room temperature, the reaction solution was dialyzed with an excess amount of a triethylamine/acetate buffer using a dialysis membrane (Slide-A-Lyzer MWCO 3 kDa, Thermo Fisher Scientific, Waltham, MA) for 14 days, followed by freeze-drying.

Biotin-conjugated MPC polymer was prepared as follows. Amine-modified MPC polymer (64 mg) was dissolved in a triethylamine/acetate buffer (pH 8, 0.1 M, 8 mL). A dimethylformamide solution (2 mL) containing 25 mg of biotin-PEG_2-NHS was added to the amine-modified MPC polymer solution. After 24 h at room temperature, the reaction solution was dialyzed with an excess amount of diluted water using a dialysis membrane (Slide-A-Lyzer MWCO 3 kDa, Thermo Fisher Scientific, Waltham, MA) for 2 days, followed by freeze-drying.

2.1. Electrospray

The coaxial electrospray (NF-102, MECC Co., Ogori, Japan) experimental equipment consisted of syringe pumps, a stainless steel outlet comprising an inner needle nested inside an outer needle, and a high-voltage generator. Typically, the inner needle was loaded with an aqueous solution (pH 4.0) containing calcium chloride (5.0 wt%), chitosan (2.0 wt%), and acetic acid (200 mM), and the outer needle was loaded with an ethanol solution containing MPC polymer (0.1, 0.2 and 0.5 wt%) and TRITC-labeled MPC polymer (0.005 wt% for visualization experiments). The solutions were simultaneously sprayed from the inner and outer needles (cathode), into an oppositely charged stainless steel dish (anode) containing an aqueous solution (receiving solution, 7 mL, pH 8.9) of disodium hydrogen phosphate (5.0 wt%) for 3 min to form CaP/chitosan microparticles covered with MPC polymer (CaP/chitosan/MPC microparticles). During electrospraying, the aqueous solution in the dish was gently stirred continuously using a magnetic stir bar (at approximately 100 rpm). The CaP/chitosan microparticles without MPC polymer were prepared by the same procedure but omitting MPC polymer.

The feed rates for both the inner and outer solutions were set at 0.2 mL/h, and the working voltage was 23 kV. The distance from the needle to the collector was 5.0 cm. The inner and outer diameters of the inner needle were 330 and 630 μm, respectively, and those of the outer needles were 1.0 and 2.5 mm, respectively.

After electrospraying, a microparticle suspension (7 mL) was left to allow precipitation. The supernatant was replaced with phosphate buffered saline (PBS, pH 7.4) and was left to allow precipitation. This washing procedure was repeated twice and, finally, the microparticles were dispersed in PBS (pH 7.4).

2.2. Characterization of CaP/chitosan/MPC microparticles

CaP/chitosan/MPC microparticles were observed using an inverted microscope (IX71, Olympus Co., Tokyo, Japan) and a confocal laser scanning microscope (CLSM) (FV1000-D, Olympus Co, Tokyo, Japan). Based on the microscope images, the diameters of 100 microparticles were measured.

The crystalline state of the microparticles was characterized by X-ray diffraction (XRD) (SmartLab, Rigaku, Tokyo, Japan) with Cu-Kα incident radiation.

2.3. Evaluation of protein adsorption onto CaP/chitosan/MPC microparticles

FITC-BSA was dissolved in PBS (pH 7.4) to prepare a 1 mg/mL solution. After mixing 500 μL of a CaP/chitosan/MPC microparticle suspension (or CaP/chitoan microparticle suspension) with 500 μL of FITC-BSA solution, the mixture was incubated for 1 h at 37 °C. The concentration of FITC-BSA in the supernatant was measured using a fluorescence spectrometer (JASCO, Tokyo, Japan) (excitation

Please cite this article in press as: T. Matsuura, T. Maruyama, Calcium phosphate-polymer hybrid microparticles having functionalized surfaces prepared by a coaxially electrospray technique, Colloids Surf. A: Physicochem. Eng. Aspects (2016), http://dx.doi.org/10.1016/j.colsurfa.2016.10.036