Polymer 55 (2014) 1143–1149

Contents lists available at ScienceDirect

Polymer

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

Uniform hollow-structured poly(vinyl amine) hydrogel microparticles with controlled mesh property and enhanced cell adhesion

Joo-Hyun Han [a], Junoh Kim [b], Shahinur Acter [c], Youngsun Kim [a], Han-Na Lee [a], Hee-Kyung Chang [b], Kyung-Do Suh [a,**], Jin Woong Kim [c,d,*]

[a] Division of Chemical Engineering, College of Engineering, Hanyang University, Seoul 133-791, Republic of Korea
[b] Amore-Pacific Co. R&D Center, 314-1 Bora-dong, Giheung-gu, Yongin-si, Gyeonggi-do 446-729, Republic of Korea
[c] Dept. of Bionano Technology, Hanyang University, 55 Hanyangdaehak-ro, Sangnok-gu, Ansan, Gyeonggi-do 426-791, Republic of Korea
[d] Dept. of Applied Chemistry, Hanyang University, 55 Hanyangdaehak-ro, Sangnok-gu, Ansan, Gyeonggi-do 426-791, Republic of Korea

ARTICLE INFO

Article history:
Received 23 March 2012
Received in revised form
4 January 2014
Accepted 14 January 2014
Available online 30 January 2014

Keywords:
Hydrogel microparticles
Mesh size
Cell adhesion

ABSTRACT

This study introduces a useful approach for fabrication of hollow-structured hydrogel microparticles and for encapsulation of biomacromolecules in the hollow core of the particles. Monodisperse hollow-structured poly(vinyl amine) hydrogel particles were fabricated without using templates that combines the dispersion polymerization and the sequential hydrolysis/crosslinking. The hydrogel shell showed pH-dependent mesh sizes; ~2 nm at a normal condition (pH 3–12) and ~11 nm at an expanded condition (pH 2). By taking advantage of pH-responsive mesh property, we demonstrated that dextran macromolecules, whose hydrodynamic radius is between the mesh sizes of the normal and expanded pH conditions, could be encapsulated and stored inside of the shell. Moreover, our hydrogel particles showed strong adhesion to human cells. Some of them were even engulfed by the cell membrane and drawn into the cell even with no aid of site-specific moieties. From these results, it is expected that the hydrogel hollow microcapsules synthesized in this study could be used for delivery of macromolecules into the cells.

1. Introduction

Microcapsules with a vesicular structure are strongly demanding to achieve a sufficient encapsulation efficiency of active ingredients and their controlled releasing [1,2]. Most well-known vesicular capsules are liposomes [3–5], polymersomes [6,7], and colloidosomes [8,9]. Each of those vesicle materials has the water-filled core enclosed by a thin hydrophobic molecular or a colloidal membrane. The liposome is made of a lipid membrane with a thickness of a few nanometers. Its membrane rigidity is relatively poor, so that wider uses for complex formulations including surfactants and other additives have been limited. To solve this, there have been efforts to fabricate vesicles with polymer amphiphiles that can lead to formation of a thicker polymer membrane with improved mechanical stability [10,11]. Also, depending on the porosity of the vesicle membrane, the level of permeation can be controlled; for example, loading and releasing of macromolecules whose molecular weights are higher than thousands of kilodalton has been regulated through the interstice of colloidosomes generated after colloidal assembly at the liquid–liquid interface [8]. This means that the mesh property should be exactly controlled to gain a tunable permeability of the vesicular membrane, consequently determining encapsulation efficiency as well as releasing property of encapsulates.

Differing from conventional vesicular capsules fabricated by means of molecular or colloidal self-assemblies, the vesicle having also a hollow structure can be fabricated by applying the layer-by-layer (L-b-L) deposition method. The polyelectrolyte membrane generated by the electrostatic interaction between polyanions and polycations is indeed flexible but rigid. It also has a water-filled core enclosed by a thin shell. This capsule system is advantageous for controlling the pore size of the polyelectrolyte membrane; pH of the continuous phase changes the pore size. [12,13] A parallel work has showed that a proteolyic enzyme could be entrapped into the polyelectrolyte microcapsules by simply adjusting pH. The enzyme in the capsule is stable against the applied heat and prolongs its initial enzymatic activity [14]. This provides a useful way to

* Corresponding author. Dept. of Bionano Technology, Hanyang University, 55 Hanyangdaehak-ro, Sangnok-gu, Ansan, Gyeonggi-do 426-791, Republic of Korea. Tel.: +82 31 400 5499; fax: +82 31 400 5457.
** Corresponding author. Tel.: +82 2 2220 0526; fax: +82 2 2220 4680.
E-mail addresses: kdsuh@hanyang.ac.kr (K.-D. Suh), kjwoong@hanyang.ac.kr (J.W. Kim).

0032-3861/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymer.2014.01.017

incorporate macromolecules into a vesicle and to release them by changing the pore size of the membrane.

Biomacromolecules, including proteins, enzymes, polysaccharides, and other biopolymers, are ubiquitous for a broad spectrum of biological and biomedical applications, including catalytic reactions, immune-assaying, biosensing, and therapeutic treatments [15–18]. However, their three dimensional molecular structures are vulnerable by environmental shocks; protein molecules are easily denatured by heat, ionic stress, and specific adhesions. Thus, appropriate formulations are needed to preserve their stability irrespective of environmental factors. This has led to development of a variety of techniques, such as DNA shuffling, mutagenesis, molecular immobilization, nano- or micro-encapsulations [19–25]. In view of practical applications, use of encapsulation techniques is favored as they can not only effectively immobilize macromolecules to maintain their original functions, but also isolate them from surrounding environment.

Here, we introduce a useful means for fabrication of hollow-structured hydrogel microparticles that have ability to encapsulate macromolecules. Hydrogel systems are intriguing, since they are biofriendly and have a controllable mesh property in response to external triggering stresses [26,27]. In this study, uniform-sized hollow-structured poly(vinyl amine) (PVAm) hydrogel particles are fabricated through simultaneous hydrolysis and disconnection of crosslinked poly(N-vinyl formamide) (PNVF) microparticles, and consecutive crosslinking of the primary amine distributing at the periphery of PVAm hydrogel microparticles. The essence of our approach lies in the fact that diffusion-limited reactions, occurring only at the peripheries of hydrogel particles, lead to the formation of hydrogel hollow microparticles with a constant shell thickness [28,29]. Using these PVAm hydrogel hollow particles, we characterize the permeation behavior of macromolecules with different hydrodynamic radii through the hydrogel shell. Finally, we carry out cell study to experimentally show that our hydrogel hollow particles are safe to the cells and can adhere to or rather penetrate into the cell membrane.

2. Experimental

2.1. Synthesis of hollow-structured PVAm hydrogel microparticles

PVAm hydrogel hollow microparticles were synthesized as previously reported [29]. First, PNVF particles were produced by using the dispersion polymerization technique. A mixture of 19 g of N-vinyl formamide (NVF, Aldrich) and 1 g of N,N′-methylene-bis-acrylamide (MBA, Aldrich) were dissolved in 170 g of methanol containing a 2,2′-azobis(isobutyronitrile) (AIBN, Junsei, 0.2 g) and poly(2-ethyl-2-oxazoline) (PETOZO, $\sim 5 \times 10^4$ Da, Aldrich, 2.4 g). Polymerization was carried out at 70 °C for 24 h with a stirring speed of 100 rpm. After polymerization, all unreacted monomers and additives were completely removed by repeated centrifugation with an ethanol. The average diameter of PNVF particles was 2.1 μm. Then, the PNVF particles (1 g) were re-dispersed in a 76 mL ethanol solution containing glutaraldehyde (GA, 50 wt% in water, Tokyo Chemical Industry). The concentration of glutalaldehyde was controlled to 0.1 mol L^{-1}. While stirring the PNVF particle dispersion, 40 g of a 2 N sodium hydroxide aqueous solution was slowly added and the reaction was carried out at 80 °C for 12 h. After washing away by-products, uniform PVAm hydrogel hollow particles were obtained.

2.2. Characterization of hydrogel hollow microparticles

The particle size of the PVAm hollow particles was determined by analysis of more than 100 particles in microscope images. The swelling ratio of hydrogel particles was observed in the solutions with different pH. To confirm their microstructure, the hydrogel capsules were labeled with a fluorescent dye. 0.2 mL of FITC solution (2 mg/mL in DMSO) was added to the capsule dispersion (0.2 mL), then diluted with water (1.1 mL). The mixture shaken for 4 h under light-shielded conditions was washed by repeated centrifugation with water. The particle shape and size were analyzed with a confocal laser scanning microscope (CLSM, BX51TRF, Olympus, Japan). CLSM was equipped with a mercury lamp and a filter cube for BX2/IX2 (excitation filter BP510-550, Dichromatic mirror DM570, Emission filter LP590). To remove the effect of ionic strength while measuring the swelling ratio at different pH, the ionic strength was adjusted to 0.001 M with KCl. The theta temperature of PVAm polymer in water was determined with a linear PVAm homopolymer. To prepare this polymer, a complete hydrolysis reaction of PNVF particles was carried out in the absence of GA. Since there was no crosslinking, the linear PVAm polymer could be dissolved in the aqueous solution. Viscosity was measured using the Ubbelohde viscometer (No. 1B) in the polymer concentration ranging from 1 to 6 g dL^{-1}. By putting the polymer solution in an isothermal water bath, the solution temperature was controlled. The zeta-potential of the particles was determined by a dynamic light scattering instrument (DLS, ELS-8000, Otsuka Electronics, Japan).

2.3. Permeation study

The permeability of biomacromolecules through the hydrogel shell was characterized by using fluorescein isothiocyanate-tagged dextran macromolecules (FITC-dextran, Sigma–Aldrich) as proxy molecules. Dried hydrogel particles (0.01 g) were re-dispersed in an aqueous solution (35 mL, pH 7). After reaching an equilibrium state, a dispersion of hydrogel particles (5 mL) was mixed with the same volume of an aqueous FITC-dextran solution (0.002 g, pH 7). We used two FITC-dextran molecules, 4 kDa and 40 kDa. Then, fluorescence from FITC-dextran in the particle dispersion was imaged using a CLSM. In the case of evaluating a long-term stability, both of dextran molecules were encapsulated at pH 2, respectively. After diffusion of dextran into the hydrogel hollow particles, pH of the solution was recovered to pH 7, thus collapsing the hydrogel phase. Then, fluorescence intensity of the particles was observed with a CLSM.

2.4. Cell culture

Normal human keratinocytes (HaCaT) were maintained in DMEM medium (Lonza) supplemented with 1% L-glutamine and 10% fetal bovine serum (FBS) (Lonza). They were grown in a humidified atmosphere of 5% CO_2 at 37 °C. Normal human dermal fibroblast cells (adult, purchased from Cascade Biologics in USA) were cultured at 37 °C in 5% CO_2 incubator with the Medium 106 with Low Serum Growth Supplement (Cascade Biologics, LSGS, cat. No. S-003-10). Both cell culture media were changed every two days.

2.5. Cell viability assay

Normal human keratinocytes (HaCaT) and normal human dermal fibroblasts were seeded in 96-well culture plates (2×10^3 cells/well) and incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 24 h. Then, the cells were cultured with hydrogel particles with varying their concentrations for 24 h. After washing with PBS twice, cell viability was measured by WST-1 assay. 10 μl of reconstituted WST-1 reagent (Roche, 11 644 807 001) and 100 μl of original culture medium were added into each well of 96-well to