Reactive & Functional Polymers 87 (2015) 7–14

Contents lists available at ScienceDirect

Reactive & Functional Polymers

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

Adsorption and recognition of protein molecular imprinted calcium alginate/polyacrylamide hydrogel film with good regeneration performance and high toughness

Kongyin Zhao [a,b,*], Tian Chen [b], Beibei Lin [b], Wenkui Cui [b], Bohong Kan [c], Ning Yang [b], Xiangyu Zhou [a], Xinxin Zhang [b], Junfu Wei [a,b]

[a] State Key Laboratory of Hollow Fiber Membrane Materials and Processes, Tianjin Polytechnic University, Tianjin 300387, China
[b] School of Material Science and Engineering, Tianjin Polytechnic University, Tianjin 300387, China
[c] The First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China

ARTICLE INFO

Article history:
Received 24 November 2014
Accepted 15 December 2014
Available online 22 December 2014

Keywords:
Protein molecular imprinting
Calcium alginate/polyacrylamide
Hydrogel film
Adsorption
Recognition
Cell culture

ABSTRACT

Protein imprinted calcium alginate/polyacrylamide hydrogel film (CA/PAM MIP) with high toughness was prepared using bovine serum albumin (BSA) as template molecule, sodium alginate and acrylamide as functional monomers, N,N′-methylenebisacrylamide (MBAA) as the covalent cross-linker and $CaCl_2$ as the ionic cross-linker via UV radiation-reduced polymerization. Factors affecting the adsorption capacity and imprinting efficiency of the BSA-imprinted CA/PAM hydrogel films were investigated, such as ratio of polyacrylamide/sodium alginate, film thickness, MBAA concentration and $CaCl_2$ concentration. Results showed that the CA/PAM MIP exhibited an obvious improvement in terms of adsorption capacity for BSA compared with non-imprinted polymer (NIP). The adsorption capacity of MIP for BSA reached 22.49 mg/g, which was 2.7 times higher than NIP. The regeneration property of the BSA-imprinted CA/PAM hydrogel was distinctly improved and the imprinting efficiency of CA/PAM MIP maintained 77.95% of the initial value after five repetitions. Single and binary proteins rebinding indicated that the CA/PAM MIP exhibited good recognition performance. Cell culture experiments showed CA/PAM MIP was more suitable for cell culture than CA/PAM NIP. The residual sodium dodecyl sulfate (SDS) in the elution process leaded to the death of mouse fibroblast cells (L929) after 3 days. A moderate elution solution without residue eluent should be used to prepare MIP for cell culture.

1. Introduction

Molecule imprinting technology has been proved to be an efficient way to prepare synthetic materials bearing selective molecular recognition sites and binding pockets at the surface or inside polymer matrix. Molecularly imprinted polymers (MIPs) are characterized by their thermal and chemical stability, high specificity, ease of mass preparation, low cost and reusability, which promote their wide applications in chromatography [1], catalysis [2], sensors [3], drug release [4] and environmental protection [5,6]. The imprinting of low-molecular weight compound has been well established. Nevertheless, several challenges remain in the imprinting of bio-macromolecules, such as proteins, DNAs, and even whole cells and viruses, due to their large size, structural complexity and the flexible conformation [7–9].

Polyacrylamide (PAM) hydrogel was widely used as the matrix for the molecular imprinting of proteins [10–12]. PAM hydrogel is biocompatible and has soft and wet macroporous structure. The abundant amide functional groups in PAM can form strong interactions with peptide bonds in the proteins even in aqueous system [13]. Protein imprinted acrylamide-based polymers have already been successfully fabricated by UV or thermally induced free radical polymerization [14–22]. However, the PAM hydrogel with low cross-linking degree and poor mechanical properties, often results in low affinity and poor regeneration properties for the template [23]. The high cross-linked PAM hydrogel effectively depresses the swelling of gel and the deformation of imprinting cavities, improving the affinity of the polymer. Nevertheless, as the cross-linking density increases, the transfer of protein was restricted [24]. Addressing these problems is usually attempted by adding another component to improve the mechanical performance and decrease the swelling of the hydrogel [11–13].

* Corresponding author at: State Key Laboratory of Hollow Fiber Membrane Materials and Processes, Tianjin Polytechnic University, Tianjin 300387, China. Tel.: +86 02283955362; fax, +86 0228395055.
E-mail address: zhaokongyin@tjpu.edu.cn (K. Zhao).

http://dx.doi.org/10.1016/j.reactfunctpolym.2014.12.001
1381-5148/

Sodium alginate is a kind of natural polysaccharide found in brown algae [25]. It consists of mannuronic acid (M) and guluronic acid (G) groups, in which G sequences can complex with calcium ions in aqueous media to form the 'egg box junctions'-like insoluble calcium alginate [26]. Many literatures have been reported using alginate as the matrix to achieve bio-macromolecular imprinting [27–33]. However, the poor mechanical stability limited their reusability. Sun and co-workers [34] fabricated calcium alginate/polyacrylamide (CA/PAM) hybrid hydrogel with excellent mechanical performances by mixing covalently cross-linked acrylamide (AM) and ionically cross-linked sodium alginate (SA). Given that the hybrid hydrogels are stretchable and tough, they may exhibit good performance in maintaining the imprinted cavities and improving the regeneration properties of the hydrogels.

In this research, a novel protein imprinted hybrid hydrogel film was synthesized through radical polymerization of AM and SA in an aqueous system followed with the cross-linking of calcium ions, using BSA as the model template, N,N′-methylenebisacrylamide (MBAA) as the covalent cross-linker and $CaCl_2$ as the ionic cross-linker. The hydrogel film was transparent, smooth, and homogeneous and its thickness could be easily controlled in the range of 0.1–0.4 mm. The adsorption and recognition properties of BSA imprinted CA/PAM hydrogel films were evaluated. The imprinting efficiency of CA/PAM MIP tended to be stable after three cycles and maintained 77.95% of the initial value after five repetitions. Single and binary proteins rebinding indicated that the CA/PAM MIP exhibited good recognition performance. Mouse fibroblast cells (L929) were cultured on the CA/PAM MIP and NIP, and the results showed that the CA/PAM MIP surface can absorb more BSA, which is helpful for the adhesion of cells. There was residual sodium dodecyl sulfate (SDS) left in the hydrogel, which leaded to the death of L929 cells after 3 days.

2. Experimental

2.1. Materials

Acrylamide (AM), N,N′-methylenebisacrylamide (MBAA) and ammoniumpersulfate (APS) were purchased from Kermel Chemical (Tianjin, China). Sodium dodecyl sulfate (SDS) and glacial acetic acid (HAc) were purchased from Institute of Guangfu Fine Chemicals (Tianjin, China). Sodium alginate (SA) was obtained from Sinopharm Chemical Reagent co., Ltd. Bovine serum albumin (BSA), ovalbumin (Ova), bovine hemoglobin (Hb) and bovine γ-globulin (Glo) were purchased from Shanghai Science and Technology Development Company (Shanghai, China). Calcium chloride dehydrate was supplied by Yingda Chemicals (Tianjin, China). Mouse fibroblast cells (L929) were obtained from the Cellular Biology Institute of the Chinese Academy of Sciences (Shanghai, China).

2.2. Preparation of CA/PAM MIP

Fig. 1 shows the schematic representation for the fabrication of CA/PAM MIP. Unless otherwise stated, the water content was fixed at approximate 86 wt%. The mass ratio of AM to SA was 6:1, 8:1, 10:1 and 12:1, respectively. The weight of MBAA and APS was fixed at 0.09and 1% of acrylamide, respectively. AM, MBAA, SA, APS and BSA were dissolved in deionized water under the magnetic stirring at room temperature. The homogeneous mixture solution was degassed at 4 °C for 12 h. Approximate 5 g mixture solution was poured into a clean glass sheet and spread out using a glass rod intertwined with copper wire with the diameter of 0.3 mm. Afterwards, the glass sheet with the mixture solution was transferred into a transparent plastic bag, purged with nitrogen for 10 min and then sealed. The polymerization was conducted for 20 min by UV irradiation (365 nm, with an intensity of 10.0 mW cm^{-2}) at room temperature to produce the polymer hydrogel. The hydrogel was left in the plastic bag for 24 h to stabilize the reactions. Then the resulted hydrogel (SA/PAM) was rinsed repeatedly with deionized water to remove unreacted monomers and cross-linkers. The protein in the hydrogel was eluted with HAc solution (10%, v/v) containing SDS (10%, w/v) according to literature [12]. After that, the hydrogel was thoroughly washed with deionized water to remove the remnant SDS and HAc. Then BSA imprinted calcium alginate/polyacrylamide film (CA/PAM MIP) was synthesized by cross-linking the hydrogel in 1.0 wt% $CaCl_2$ for 5 h.

Non-imprinted calcium alginate/polyacrylamide film (CA/PAM NIP) was also prepared in the same way without adding BSA. BSA imprinted and non-imprinted calcium alginate (CA) films and polyacrylamide (PAM) hydrogel microspheres were also prepared according to literature [15,30].

2.3. Characterizations

The morphology of CA/PAM MIP in wet form was characterized using a digital camera. The surface morphologies of the dried CA, PAM, and CA/PAM hydrogels were recorded by SEM (FESEM; S-4800, HITACHI, Japan). Samples of CA/PAM were dried at 40 °C under vacuum to constant weight.

The mechanical properties of CA/PAM hygrogels were tested using a tensile testing machine (LLY-06F, Laizhou Electronic Instrument Co., Ltd., China).

2.4. Adsorption experiments

The adsorption of BSA on CA/PAM MIP and CA/PAM NIP was carried out using batch adsorption method. The surface water on wet hydrogel film was absorbed with filter paper, and then the hydrogel film was added into a glass bottle containing 10 mL 1.36 mg/mL BSA solution to evaluate the imprinting efficiency and dynamic adsorption. Similarly, hydrogel film was added into various BSA concentrations (0–3.0 mg/mL) to determine the adsorption isotherms. The protein concentration in the supernatant was determined by a UV spectrophotometer at 280 nm. Equilibrium adsorption capacity Q_e (mg/g) was calculated according to the following equation:

$$Q_e = (C_0 - C_e)V/m \quad (1)$$

where m (g) is the mass of wet gels, V (mL) is the volume of protein solution, and C_0 (mg/mL) and C_e (mg/mL) are the protein concentrations of initial solution and the supernatant at equilibrium, respectively. The imprinting efficiency (IE) of CA/PAM MIP was presented as follows:

$$IE = Q_{MIP}/Q_{NIP} \quad (2)$$

where Q_{MIP} and Q_{NIP} are the adsorption capacity of CA/PAM MIP and NIP.

The dynamic adsorption capacity Q_t (mg/g) at time t was calculated according to following equation:

$$Q_t = (C_0 - C_t)V/m \quad (3)$$

where C_t (mg/mL) is the BSA concentration in the supernatant at time t.

2.5. Recognition performance

Wet CA/PAM MIPs or NIPs (0.2 g) were placed into a series of bottles containing 10 mL of 1.36 mg/mL different protein solutions for 24 h to evaluate the recognition performances. The recognition performance of MIPs was evaluated by the static distribution coef-