Chinese Chemical Letters 26 (2015) 807–810

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Polypropylene non-woven supported fibronectin molecular imprinted calcium alginate/polyacrylamide hydrogel film for cell adhesion

Dun-Wan Zhu [a], Zhuo Chen [a], Kong-Yin Zhao [b], Bo-Hong Kan [c], Lan-Xia Liu [a], Xia Dong [a], Hai Wang [a], Chao Zhang [a], Xi-Gang Leng [a], Lin-Hua Zhang [a,*]

[a] Institute of Biomedical Engineering, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin Key Laboratory of Biomedical Materials, Tianjin 300192, China
[b] State Key Laboratory of Separation Membranes and Membrane Processes, 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 12 February 2015
Received in revised form 10 April 2015
Accepted 23 April 2015
Available online 5 May 2015

Keywords:
Molecular imprinting film
Calcium alginate/polyacrylamide
Fibronectin
Cell adhesion

ABSTRACT

Fibronectin (FN) imprinted polypropylene (PP) non-woven supported calcium alginate/polyacrylamide hydrogel film (PP-s-CA/PAM MIP) was prepared using non-woven PP fiber as matrix, FN as template molecule, sodium alginate (SA) and acrylamide (AM) as functional monomers, *via* UV radiation-reduced polymerization. The PP-s-CA/PAM MIP exhibited an obvious improvement in terms of adsorption capacity for FN compared with non-imprinted polymer (NIP). The PP-s-CA/PAM MIP was successfully used for the culture of mouse fibroblast cells (L929) and the results showed that PP-s-CA/PAM MIP exhibited better cell adherence performance than the NIP did.

1. Introduction

Biomaterials with the potential to recognize specific molecules have received considerable attention in the fields of bioscience and bioengineering. The biomolecules including protein antibodies and enzymes are often used as signal-capturing moieties to develop biological substitutes for the therapeutic replacement and tremendous need for organs and tissues [1]. However, these biomolecules are not very stable, they cost a lot and their affinity reduces significantly under artificial conditions [2,3]. Molecular imprinting is a technique involving artificial recognition sites that can memorize the shape and chemical properties of the target molecules. Compared to the affinity matrices prepared using antibodies or enzymes, the molecularly imprinted polymers (MIP) possess unique properties, such as high stability, low cost, and easy preparation [3,4]. The MIPs have been used in various applications including separations [5], solid-phase extraction, catalysis [6], biomimetic sensor [7], and drug delivery [8]. Pan *et al.* [9] employed a molecular imprinting methodology to introduce the cell-adhesive peptide RGDS onto a thermo-responsive cell culture substrate, which was innovatively used as a highly efficient novel system for harvesting cell sheets. Fukazawa *et al.* [10] proposed a new molecular imprinting procedure for the purpose of cell capture using the cell-adhesive protein fibronectin (FN) as the template. The binding of FN from the cell culture medium with the fetal calf serum was achieved on the FN imprinting substrate, and induced the cell adhesion.

The imprinting of low-molecular weight compounds has been well established. However, several challenges remain in the imprinting of proteins due to the large size, the structural complexity and the flexible conformation of the bio-macromolecular [11]. Polyacrylamide (PAM) hydrogels have been regarded as suitable imprinting matrices for proteins [12]. But the poor mechanical property and poor regeneration of the soft texture limited its application. Bovine serum albumin (BSA) imprinted polypropylene (PP) fiber-grafted polyacrylamide hydrogel was prepared using non-woven PP fiber as matrix, BSA as template molecule, and acrylamide as functional monomer [13]. In order to improve the strength of the PAM hydrogel, BSA imprinted calcium alginate/polyacrylamide hydrogel film was prepared by using BSA as template, acrylamide and sodium alginate as functional monomers. Acetic acid solution containing sodium dodecyl sulfate (SDS) was used to remove BSA. However, it is different to completely remove SDS and the residual SDS can lead to the death of cells [14]. In this paper, PP non-woven supported FN imprinted calcium alginate/polyacrylamide hydrogel membrane

* Corresponding author.
E-mail address: elley2001@sina.com (L.-H. Zhang).

http://dx.doi.org/10.1016/j.cclet.2015.04.033

(PP-s-CA/PAM MIP) was prepared using FN as template molecule. A moderate elution solution was used to remove the template FN, and the resulted PP-s-CA/PAM MIP was used for the culture of mouse fibroblast cells (L929).

2. Experimental

2.1. Materials

Non-woven polypropylene (PP) fiber (22 g/m^2) was obtained from Xianghehuaxin Non-woven Company, LTD (Langfang, China). Sodium alginate (SA) was purchased from Tianjin Yuanhang chemical company. Acrylamide (AM) and *N,N′*-methylenebisacrylamide (MBA) was analytical reagent and purchased from Chemistry Reagent Factory of Tianjin (Tianjin, China). $CaCl_2$ was supplied by Yingda Chemicals (Tianjin, China). Trihydroxymethyl aminomethane (Tris) and ammonium persulfate (APS) was obtained from the Institute of Tianjin Guangfu Fine Chemicals (Tianjin, China). Fibronectin (FN, 440KD) was purchased from Shanghai Qianchen Biological Technology Company (Shanghai, China). Mouse fibroblast cells (L929) were obtained from the Cellular Biology Institute of the Chinese Academy of Sciences (Shanghai, China).

2.2. Preparation of PP-s-CA/PAM MIP

Fig. 1 shows the schematic representation of the fabrication procedure of the FN-imprinted PP-s-CA/PAM. Non-woven PP fiber (200 mg) was immersed into 10 mL aqueous solution consisting of FN (2 mg), AM (1 g), APS (10 mg), MBA (0.8 mg), and SA (50 mg). The mixture was incubated for 1 h at room temperature for the pre-assembly between the template molecules and functional monomers. Then the non-woven PP fiber immersed in the mixture was transferred into the quartz glass sheet, purged with nitrogen for 8 min to remove oxygen, and sealed. The polymerization was conducted according to the literature [15]. The hydrogel supported on non-woven PP was cross-linked with 1% $CaCl_2$ for 3 h, and washed repeatedly with deionized water to remove unreacted monomer and cross-linker. The protein in the hydrogel was eluted with Tris–HCl buffer solution (pH 8.3). Then FN imprinted PP non-woven supported calcium alginate/polyacrylamide hydrogel film (PP-s-CA/PAM MIP) was prepared after extensively washed with deionized water to remove remnant Tris–HCl buffer solution.

The non-imprinted polymer was synthesized by using the same procedure in the absence of template molecule and was noted as PP-s-CA/PAM NIP.

2.3. Characterization

The morphologies of PP-s-CA/PAM MIP were observed using a scanning electron microscope (FESEM; S-4800, HITACHI, Japan).

2.4. Protein adsorption

About 0.2 g of wet PP-s-CA/PAM MIP or NIP (using filter to absorb the surface water) was placed in a glass bottle containing 5 mL 0.2 mg/mL FN solution. At specific time intervals, samples were withdrawn from the supernatant and determined with a UV spectrophotometer. The adsorption capacity Q (mg/g) of the protein adsorbed onto PP-s-CA/PAM MIP or NIP was calculated according to the literature [14].

2.5. Cells adhesion and proliferation on PP-s-CA/PAM MIP and NIP

The PP-s-CA/PAM MIP and NIP were sterilized by rinsing with ethanol and then inserted into a 24-well tissue culture plate. L929 cells were seeded onto PP-s-CA/PAM MIP and NIP at a density of 2×10^4 cells/well and cultured at 37 °C under a humidified atmosphere of air containing 5% CO_2. At the desired time point, the viability of cells cultured on the MIP and NIP was observed by microscopy (OLYMPUS IX71, Japan). The adhesion behavior of L929 cells was researched according to the literature [9].

3. Results and discussion

Fig. 2 shows the digital (in 24-well tissue culture plate containing deionized water) and SEM images of PP-s-CA/PAM MIP. The digital photo of PP non-woven was also shown on the left side of Fig. 2(a). The CA/PAM hydrogel was hydrophilic and transparent, so the PP-s-CA/PAM MIP was translucent. PP non-woven was hydrophobic and some air bubbles were trapped in the PP fiber when it was immersed in the water, so the PP non-woven was opaque. Significant lamellar macroporous structure was found on the surface of PP-s-CA/PAM MIP (Fig. 2(b)). The macroporous structure on the surface of MIP facilitated the diffusion of the protein template. But the imprinting pores were not found because

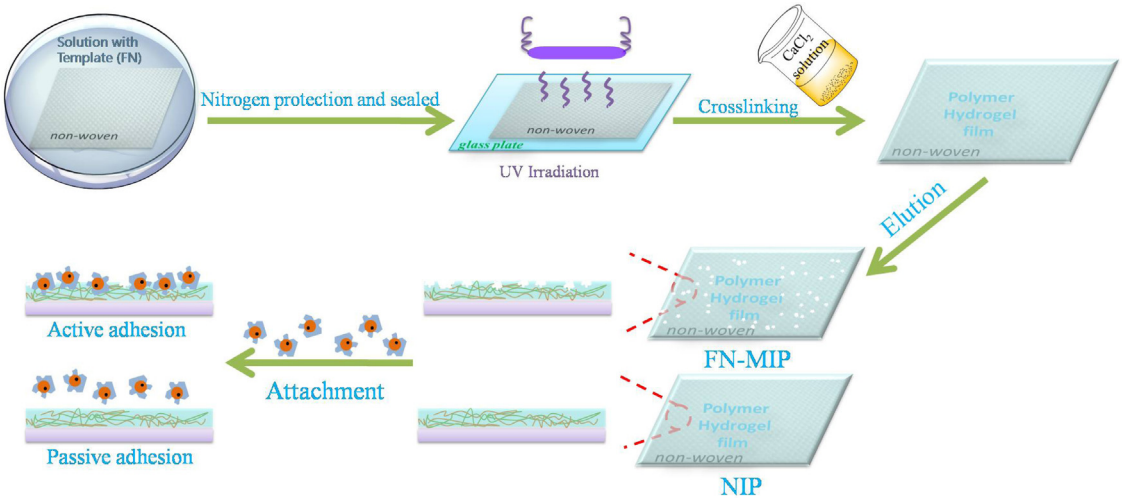

Fig. 1. Schematic representation of the fabrication procedure of imprinted PP-s-CA/PAM and cell adhesion.