Journal of Chromatography A, 1359 (2014) 330–335

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Short communication

Quality improvements of cell membrane chromatographic column

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ARTICLE INFO

Article history:
Received 9 February 2014
Received in revised form 21 July 2014
Accepted 23 July 2014
Available online 1 August 2014

Keywords:
Cell membrane chromatography
Methodology optimization
HSC-T6
SMMC-7721
Quality improvement
Paraformaldehyde

ABSTRACT

Cell Membrane Chromatography (CMC) is a biological affinity chromatographic method using a silica stationary phase covered with specific cell membrane. However, its short life span and poor quality control was highlighted in a lot of research articles. In this study, special attention has been paid to the disruption, cell load and packing procedure in order to improve the quality of the CMC columns. Hereto, two newly established CMC models, HSC-T6/CMC and SMMC-7721/CMC have been developed and used in this research project. The optimization of the abovementioned parameters resulted in a better reproducibility of the retention time of the compound GFT (RSD < 10%) and improved significantly the quality of the CMC columns. 3.5×10^7 cells were the optimal cell load for the preparation of the CMC columns, the disruption condition was optimized to 5 cycles (400 W and 20 s interval per cycle) by an ultrasonic processor reducing the total time of cell disruption to 1.5 min and the packing flow rate was optimized by applying a linear gradient program. Additionally, 4% paraformaldehyde (PFA) was employed to improve the column quality and prolong the column life span. The results showed that the retention time was longer with PFA treated columns than the ones obtained with the control groups

1. Introduction

Cell Membrane Chromatography, developed by He et al. and improved by several groups, has been widely used as an effective method to screen potential effective drugs or components [1,2]. Its applications are well accepted in research projects on interactions between membrane receptors and potential drugs, as well as in competitive binding experiments [3,4]. As a bioactive model, CMC has shown a great potential in screening active components both offline and online [5]. The principle of this model is clear. In brief, the bioactive membrane fragments were collected and incubated with silica to be adsorbed to form a cell membrane stationary phase (CMSP). Then the CMSP was packed into a column to build up a CMC model [6]. Since the membrane covered on the silica is bioactive and the fact that column can be used under routine conditions, CMC column combines the advantages of both biomaterials and classic chromatography to realize online and high-throughput screening of potential active drugs from complex matrices [7]. For years, massive potential drugs have been screen by this method, some of which have even been applied into clinical research stages [8–10].

Although CMC has been widely used, no common standard to measure the quality of CMC column is available. The short column life span and the variety among the different batches also prohibit CMC from producing stable and reliable results. According to the literature, the activity loss or falling off of the cell membrane protein on CMSP can be considered as a key factor for the short life span of the column [11]. These problems may affect the column quality and the screening results.

In this study, two CMC models, HSC-T6/CMC and SMMC-7721/CMC, have been developed and optimized. Based on the previously reported procedure [6] and a new cell disruption strategy, several variables such as cell load, disruption conditions, packing procedures and the use of the fixative paraformaldehyde (PFA) [12] have been fully investigated to improve the quality of the CMC methodology with the option to obtain more reliable results.

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http://dx.doi.org/10.1016/j.chroma.2014.07.071
0021-9673/

2. Experimental

2.1. Materials and instruments

Gefitinib (GFT) and Tetracycline (TC) were purchased from Nanjing Ange Pharmaceutical Co., Ltd. (Nanjing, China). The standard solution of GFT (10 mM) and TC (10 mM) were prepared in methanol and water respectively. The purity of all the standard compounds was more than 99.5%. Dulbecco's modified Eagle's medium (DMEM) and Phosphate buffer saline (PBS) were purchased from Hyclone (Thermo Fisher). Fetal bovine serum (FBS) was obtained from Gbico Life Technology Co. (Australia). Dimethyl sulfoxide (DMSO), penicillin streptomycin and trypsin were purchased from Gibco-BRL Co. (Rockville, MD, USA). Silica gel (3 μm, spherical, 200 Å) was obtained from Melone Pharmaceutical Co., Ltd. Paraformaldehyde (PFA) was purchased from Bio-Light Biotech (Shanghai, China). RIPA (Radio Immunoprecipitation Assay) lysis buffer was purchased from Beyotime Co. (Shanghai, China). Ultrapure water was prepared by a Milli-Q Academic A10 water purification system (Millipore, Bedford, MA, USA). Other reagents were of analytical grade. Pipettes were purchased from Gilson, Inc. (France). The balance we used was AND HA-202M (Japan). The centrifuge was HITACHI CR21GIII from Hitachi Co., Ltd. (Japan). BCA protein quant assay kit was purchased from Beyontime Co. (Shanghai, China). The HPLC system was Waters-996-515 from Waters Co., Ltd. (America). The ultrasonic processor was JY92-IIN from Scientz Biotechnology (Ningbo, China).

2.2. Preparation of cell culture

Human hepatoma cell line SMMC-7721 was purchased from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology. HSC-T6 cell, an immobilized rat hepatic stellate cell line with stable phenotype and biochemical characters was kindly provided by Dr. Friedman SL (Liver Center Laboratory, San Francisco General Hospital, USA). Both cell lines were cultured in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL benzylpenicillin, and 100 μg/mL streptomycin in a humidified environment with 5% CO_2 at 37 °C. Cells from exponentially growing cultures were harvest in this experiment. Cells were stored in liquid-nitrogen before anabiosis. The characteristics of both cell lines are indicated in supplementary data.

2.3. HSC-T6/CMC model and SMMC-7721/CMC model

HSC-T6/CMC and SMMC-7721/CMC columns were prepared according to previously reported methods with slight modifications by applying a new disruption strategy and a PFA-treatment procedure [6]. Briefly, certain count of cells were harvested and then washed three times with PBS by centrifuging at 110 × *g* for 10 min. PBS was then added to produce a cell suspension. The cells were disrupted by a new strategy based on an ultrasonic processor under the optimized conditions. The resulting homogenate was centrifuged at 1,000 × *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 12,000 × *g* for 20 min. The precipitation was then suspended in 5 mL PBS. Cell membrane stationary phase (CMSP) was prepared by the adsorption of cell membrane suspension on 0.04 g silica under vacuum and agitation conditions. All the procedures above were implemented at 4 °C. After overnight-incubation for 12 h, the CMSP was washed three times with PBS by centrifuging at 110 × *g* for 5 min. The pellet was suspended in PBS and packed into the column (10 mm × 2 mm I.D., purchased from Dalian Replete Science and Technology Co., Ltd.) by an LC pump (Waters 996) with PBS. The packing flow rate was optimized as a linear gradient program as follows: 0–5 min, from 0.2 mL min^{-1} to 1.0 mL min^{-1}; and 5–5.5 min, 1.0 mL min^{-1}. Then the column is equilibrated 1 h at the flow rate of 0.2 mL min^{-1} and the temperature of 37 °C until stable column pressure and baseline have been obtained. The CMC columns were stored in PBS at 4 °C.

2.4. Optimization of cell disruption

The ultrasonic condition was optimized to achieve proper cell disruption. Briefly, 3.5×10^7 HSC-T6 cells or SMMC-7721 cells were ultrasonicated for 2 s at the power of 400 W followed by a 20 s cooling period, and this process was repeated for 1, 3, 5 or 7 cycles. The resulting suspension was imaged at 40× magnification with an Olympus IX71 microscope. Then the suspension was centrifuged at 1,000 × *g* for 10 min. Supernatant was collected to be centrifuged at 12,000 × *g* for 20 min to harvest the lysed membrane. The pellet was suspended in 5 mL PBS to determine the concentration of membrane protein by BCA protein quant assay kit. Cells at the load of 0.5×10^7 and 5×10^7 were also investigated.

2.5. Optimization of cell load

The cell load required to prepare per CMC column was optimized to ensure the quality of CMC columns. Different load of HSC-T6 cells or SMMC-7721 cells varying from 0.5×10^7 to 5×10^7 were used to prepare a CMC column. Total quant of membrane protein was used as an indicator to assess the quant of cell membrane. The concentration of membrane protein was examined by BCA protein quant assay kit before the overnight-incubation (12 h) of CMSP. Then the CMSP was washed with PBS 3 times by centrifuging at 110 × *g* for 10 min to eliminate the surplus membranes. The resulting pellet of CMSP was suspended in RIPA to determine the quant of membrane adsorbed on it after overnight-incubation(12 h) by BCA protein quant assay kit.

Different load of HSC-T6 cells or SMMC-7721 cells varying from 0.5×10^7 to 5×10^7 were also processed to make cell membrane columns. The quality of these columns is validated by the calculation of the reproducibility of the retention time of marker ligand ($n = 6$).

2.6. Treatment of CMC columns with PFA

Cell membrane coated on the CMSP was tentatively fixed by 4% PFA for the first time to stabilize the membrane adsorbed on the silica and thus to improve the quality of CMC column. Both HSC-T6/CMSP and SMMC-7721/CMSP were examined. Immediately after the CMSP were washed and prepared, they were divided into PFA-treated group and control group. The two groups were incubated with 4% PFA and PBS for 30 min at 37 °C respectively, and centrifuged at 1,000 × *g* for 10 min. The precipitation was washed with PBS and was centrifuged again. The supernatant was discarded and the CMSP was ready for being packed into columns.

2.7. CMC analysis

CMC analysis was performed on a Waters 996-515 HPLC system consisting of two unitary pumps, a thermostatically controlled column oven and a diode array detector. The flow rate was 0.2 mL min^{-1} with 10 mM PBS and the injection volume was 5 μL for each run. Column temperature is 37 °C. Retention time of marker and negative ligands were recorded and analyzed by Millennium^{32} (Waters) software for 6 runs of each column.