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SHORT COMMUNICATION

Novel Cell Membrane Capillary Chromatography for Screening Active Compounds from Natural Products

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Abstract Cell membrane chromatography (CMC) is a useful method for the simultaneous isolation and identification of active compounds from natural products. However, it suffers from high cell membrane consumption and is time-consuming to operate. In this study, CMC was performed for the first time with a silica capillary, termed cell membrane capillary chromatography (CMCC). Pancreatic islet cell membranes from a mouse were immobilized onto the capillary inner wall functionalized with aldehyde groups. Scanning electron microscopy observation of the prepared column showed that the cell membrane was evenly coated onto the capillary inner wall. Three model analytes with the pharmacological property of hypoglycemic activity including glibenclamide, glipizide and berberine were tested. They were all retained by the prepared column. Furthermore, the retention factors of the analytes in CMCC correlated well with their pharmacological action. The analytical procedure including washing (to obtain a flat baseline), injection and separation was accomplished within 10 min. The CMCC column was also used for screening active compounds from a natural plant (*Coptis chinensis*). The hypoglycemia activity of active components such as berberine was verified using the method. The results indicated that CMCC is a viable alternative method for screening active compounds from natural products.

Keywords Cell membrane capillary chromatography · Berberine · *Coptis chinensis* · Analysis

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Introduction

Natural products are one of the major sources of newly discovered drugs. For example, qinghaosu (artemisinin), possessing the most rapid action of all current drugs against *Plasmodium falciparum* malaria, was isolated from the plant *Artemisia annua*. Screening of active compounds from a natural product always requires the isolation of components and measurement of their activities [1]. The methods used are often complicated and time-consuming. Highly efficient screening methods which offer both isolation of compounds and determination of their activity are therefore needed.

Cell membrane chromatography (CMC) utilizes cell membrane-immobilized silica particles as the packing material to screen the active compounds. The eluted analytes are thereafter collected and further analyzed by mass spectrometry (MS) to obtain detailed structure information. Therefore, CMC is a practical method for screening active compounds from natural products. So far, it has been systematically studied [2–11]. Previous research has identified that the activity of the compound was in good relation with the retention characteristic in CMC. Thus, this system can be used directly to imitate the interaction process between a drug (or active compounds) and the target (membrane receptor) in vivo [2]. In addition, CMC has been used to study the interactions between components in

natural products and their receptors. However, some drawbacks arise in the preparation and use of CMC. Firstly, a large number of active cells are needed to prepare the silica packing. Rabbits with more active cells than mice have been adopted to overcome this problem [6]. Besides, cultured cells were also used to provide the cell membranes for the method [10, 11]. Secondly, the overall process can be time-consuming, from packing the CMC column to performing the first injection. For example, He et al. [2] reported that it took 4 h to obtain a flat baseline before the first injection. The activity of some of the prepared cell membranes was also found to decrease with time. Some compounds which have weak interactions with the receptors on cell membranes may not be detected by the CMC method. Lastly, physical adsorption is the process by which the cell membrane is immobilized on the silica particles. Though physical adsorption is considered to be irreversible, immobilization by both physical adsorption and chemical binding is thought to be more stable than by physical adsorption alone.

In this study, CMC was performed for the first time using a silica capillary, termed cell membrane capillary chromatography (CMCC). Pancreatic islet cell membranes from a mouse were immobilized onto the capillary inner wall functionalized with aldehyde groups. Cell membranes obtained from a mouse were adequate for CMCC. The morphology, activity and repeatability of CMCC were investigated.

Experimental

Materials and Instrumentation

(3-Aminopropyl)trimethoxysilane (APTS, 97 %) and glutaraldehyde were obtained from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals were all analytical grade and obtained from local sources. Deionized water was used in all the experiments. All solvents were filtered through a 0.22-μm membrane filter. ATPase kits were bought from Jiancheng Chemical Co., (Nanjing, China). Untreated fused-silica capillaries of 50 μm ID were provided by Yongnian Optic Fiber (Hebei, China).

Chromatographic analysis was performed with a PA 800 Plus Pharmaceutical analysis system and a 32 Karat workstation (Beckman Coulter Fullerton, CA, USA). A UV-3100 UV spectrophotometer (Hitachi Co. Tokyo, Japan) was used to determine the activity of the prepared cell membrane. S-4800 scanning electron microscopy (Hitachi Co., Tokyo, Japan) was employed to observe the prepared capillary column, and a KQ-200VDE supersonic cleaner (Kunshan Supersonic Equipment Co., Jiangsu, China) was used to break cells.

Extraction of the Cell Membrane

Male Kunming mice (20 ± 2 g) were supplied by the Animal Center of Tianjin Medical University (Tianjin, China). The cell membrane of pancreatic islet cell was prepared as previously described [3, 6, 12]. Briefly, the pancreas of mouse was washed with PBS (50 mmol L^{-1}, pH 7.4) and then cut into small pieces. After that, the pancreas was digested by about 1 mL collagenase solution (1 mg mL^{-1}) at 37 °C for about 20 min. Thereafter, the dispersed cells were obtained. They were then mixed with Ficoll-400 solution and centrifuged at 7,200 rpm for 5 min at 4 °C. The obtained pancreatic cells (7×10^6) were washed thrice with normal saline solution by centrifugation for 5 min at 4 °C. Tris–HCl (50 mmol L^{-1}, pH 7.4) was then added to produce a cell suspension. The suspension was sonicated for 30 min at 4 °C to break the cells. The resulting homogenate was centrifuged at $200 \times g$ for 5 min. The obtained supernatant was then centrifuged at $15{,}000 \times g$ for 20 min at 4 °C. Thereafter, the supernatant was discarded and Tris–HCl (50 mmol L^{-1}, pH 7.4) was added to the pellet. The pellet was mixed with water, from which the cell membrane suspension was prepared. The activity of the prepared cell membrane was determined by ATPase using commercial kits (Jiancheng Chemical Co., Jiangsu, China).

Preparation of the Cell Membrane-Coated Capillary

The cell membrane was either coated onto the untreated capillary or modified onto an aldehyde group-functionalized capillary. To immobilize the cell membrane on the untreated capillary, the cell membrane suspension was poured into the capillary at 4 °C, and left overnight. Unreacted cell membrane was then washed away with the Tris–HCl buffer (pH 7.4, 50 mmol L^{-1}). The prepared capillary was filled with buffer and stored at 4 °C before use. To immobilize the cell membrane onto the aldehyde group-functionalized capillary, the capillary was pretreated by silanization with APTS and then functionalization with glutaraldehyde. The procedure was the same as that reported by Xu et al. [13]. Briefly, 20 % APTS solution dissolved in dry acetone was introduced into the pretreated capillary at room temperature for 60 min. Thereafter, the capillary was rinsed by acetone and then blown dry with nitrogen. Then, 10 % glutaraldehyde dissolved in 50 mM borate buffer (pH 9.0) was used to wash the capillary at room temperature for 60 min. The obtained capillary was flushed with water for 15 min and then transferred into the last step for cell membrane protein immobilization. The prepared capillary was then flushed with the cell membrane suspension at 4 °C for 60 min. Thereafter, the capillary was rinsed with water to wash out the unimmobilized cell

membrane and then filled with the Tris–HCl buffer for subsequent use.

CMCC Conditions

Cell membrane-coated capillaries of 60 cm effective length and 70 cm total length were used. On-column UV detection was carried out at 214 nm. The temperature of the column was controlled by the experimental instrument. It was kept at 37 °C unless otherwise stated. Berberine, glibenclamide and glipizide were dissolved separately in thrice distilled water to prepare samples of concentration 50 μg mL^{-1}. All the samples were stored at 4 °C before injection. In CMCC, a pressure of 3 psi was used and 50 mmol Tris–HCl (pH 7.4) was selected as the mobile phase. A pressure of 0.5 psi for 3 s was used for sample injections.

Preparation of the Extraction of *Coptis chinensis*

Coptis chinensis was purchased from the TCM Store (Tianjin, China) and was identified by Prof. Ye Zhou (School of Pharmacy, Tianjin Medical University). It was ground and passed through a filter (80 μm). One gram of ground sample was reflux extracted with 25 mL water for 2 h three times. The extract was then concentrated under reduced pressure, after which the sample was subjected to a C_{18} (500 mg) solid phase extract column (Agilent Technologies, CA, USA). The column was washed with 10 mL water, and then eluted a mixture of CH_3OH/H_2O (80:20 v/v) as the mobile phase, to give 15 mL eluent. The 15 mL eluent was evaporated under reduced pressure to give a residue, which was redissolved in 2 mL water and then filtered through a 0.22 μm membrane filter for further analysis.

Results and Discussion

Characterization of the Cell Membrane-Immobilized Capillary

In CMCC, cell membranes were immobilized onto a capillary inner wall, resulting in low cell membrane consumption. To demonstrate the advantage of this method, mouse pancreatic islet cells were selected for this study. Compared with other cells (like blood cells), these cells need to be prepared in more restricted conditions and tend to have a lower production rate. Thus, more cells are needed in conventional CMC. In one example to overcome this problem, rabbit pancreatic cells were adapted by Yang [6]. We prepared the CMCC column with mouse pancreatic islet cells. The average amount of membrane protein bound to the inner wall of the capillary was 1.63 ± 0.21 mg cm^{-2}. The cell membranes from one mouse have been calculated to modify a capillary (50 μm ID) of more than 50 m. This means that the cell membranes from a mouse could be used more than 70 times in this study by adopting a capillary 70 cm in length.

Cell membranes absorbed onto the silica surface by self-fusion have been reported to be stable [2]. Therefore, in our initial research, pancreatic cell membranes were coated onto the capillary inner wall by physical adsorption. However, the adsorption was found not to be stable at body temperature (37 °C). The capillary at this temperature suffered from blocking, which may have been due to the leakage of the cell membranes eluted from the capillary inner wall. To overcome the problem, aldehyde groups were introduced onto the capillary inner surface by silanization and then glutaraldehyde functionalization. N-terminals of the proteins in the cell membranes could then be chemically bonded with the capillary. Using this process,

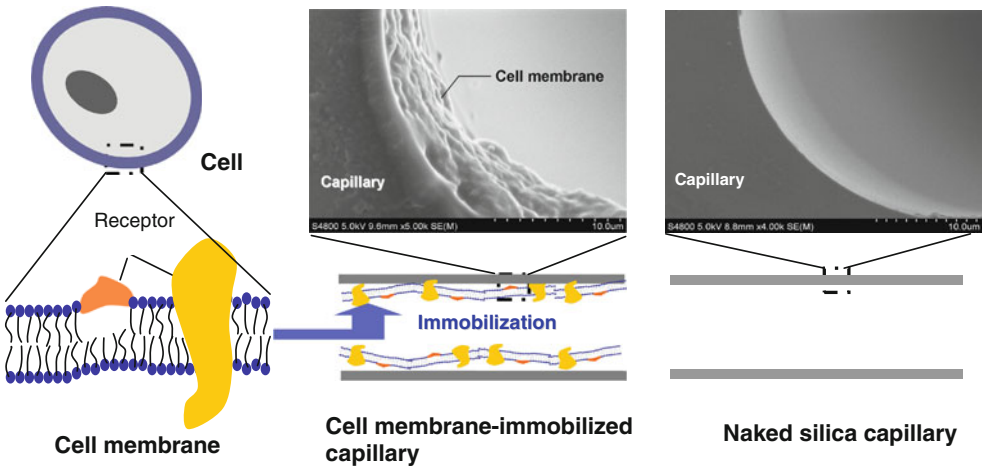

Fig. 1 Scheme of the preparation of the cell membrane-coated capillary

the cell membrane can be chemically immobilized onto the silica surface. Furthermore, the unreacted silanol groups may physically adsorb to the cell membranes. Thus, cell membrane immobilization is considered to proceed by both chemical and physical adsorption. The SEM images of the capillary were shown in Fig. 1. As can be seen, compared with the naked capillary (whose inner surface is smooth), the prepared capillary's inner surface was evenly coated by the cell membranes. The CMCC column was well tolerated at body temperature, and no column blocking was found in repeated uses.

To characterize the biological activity of the prepared column, three model analytes with anti-diabetic activity were tested. The agonist of sulfonylurea receptors such as glibenclamide and glipizide were used as positive control of synthesized drug. Berberine with anti-diabetic constituent from *C. chinensis* used as positive control of active natural compound, and DMSO was used for determination of the dead time. A bare capillary was also used for comparison. All analytes were eluted simultaneously in the bare capillary. Different retention factors for the three compounds were obtained. The results are shown in Table 1. All of the tested analytes eluted after the marker (dimethyl sulfoxide). Furthermore, the retention factors of glipizide and glibenclamide in CMCC correlate well with their pharmacological actions.

The activity of pancreatic islet cell membranes is responsible for the retention and separation of the analytes in CMCC. ATPase has been reported to reflect the activity of cell membranes [2]. In this study, the suspension of mouse pancreatic islet cell membranes' activities of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase were determined. The results showed that the activity of the Na^+–K^+ ATPase in the prepared cell membrane remained at 50 % after one day in 4 °C, and it reduced to less than 30 % at room temperature. Compared with traditional CMC, CMCC is a more time-saving method. In this research, the process of establishing equilibrium of the system is <10 min. Conversely, traditional CMC needs 4 h to achieve the same process [2].

Table 1 Elution times and retention factors of glibenclamide, glipizide and berberine

	Elution time (min)	RSD (%)	Retention factor
DMSO	3.09	1.97	–
Glibenclamide	3.15	1.72	0.019
Glipizide	3.19	1.90	0.034
Berberine	3.60	2.99	0.165

Chromatographic conditions, column (60 cm efficient length, 70 cm total length, 50 μm ID); mobile phase, 50 mmol L^{-1} Tris–HCl buffer (pH 7.4); applied pressure, 3.0 psi; detection wavelength, 214 nm; column temperature, 37 °C

The reproducibility of the prepared column was investigated by repeating the separation of the compounds 10 times with a single capillary column. The relative standard deviations of the retention times for the analytes were found to be <2.99 % (Table 1). In addition, no apparent loss of the retention factor was found in those separations. The results indicate that the column is promising for repeated use.

Use of CMCC to Screen Active Constituents from *C. chinensis*

To further demonstrate the utility of the CMCC method, active compounds from *C. chinensis* were screened by CMCC. Components of *C. chinensis* such as berberine have been found to have anti-diabetic activity [14, 15]. Analysis of the extract of *C. chinensis* and the active compound berberine by CMCC are shown in Fig. 2. The retention time of berberine was 3.60 min (Fig. 2a), and the

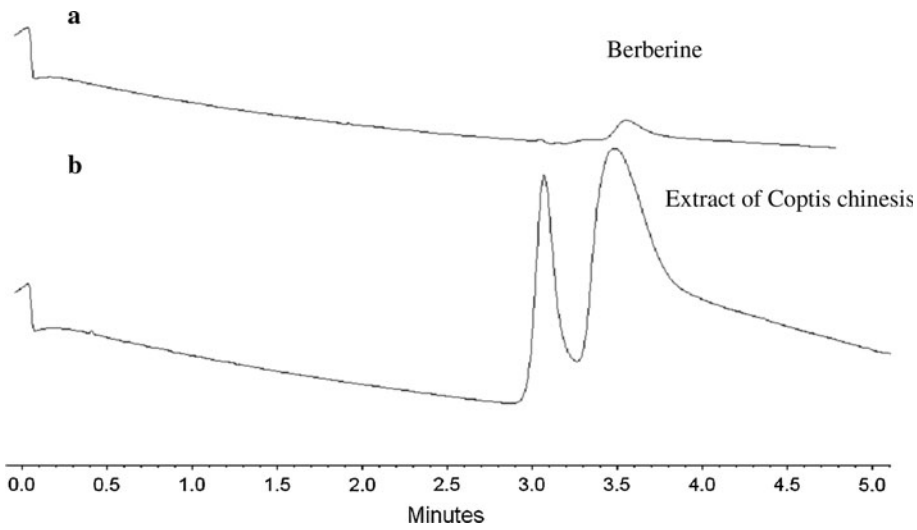

Fig. 2 Chromatographic analysis by CMCC. *a* Berberine, *b* extract of *Coptis chinensis*. Chromatographic conditions, column (60 cm efficient length, 70 cm total length, 50 μm ID); mobile phase, 50 mmol L^{-1} Tris–HCl buffer (pH 7.4); applied pressure, 3.0 psi; detection wavelength, 214 nm; column temperature, 37 °C

void time was 3.09 min (as shown in Table 1, DMSO). The retention factor of about 0.17 for berberine was therefore obtained. The sample of the plant extract exhibits two peaks (Fig. 2b). The first peak which has similar retention time with that of DMSO may have no activity, and the second peak which has similar retention time with berberine may contain the same active compound.

Conclusions

A silica capillary coated with mouse pancreatic islet cell membranes was employed for the first time for CMC. We named the method CMCC. The preparation of the CMCC column required fewer active cell membranes than conventional CMC. In this CMCC, the cell membranes were presumed to be immobilized onto the capillary inner wall by both chemical binding and physical adsorption. Blocking of the capillary by leakage of the cell membrane from the capillary was therefore avoided. In addition, CMCC was found to be more efficient for procedures such as washing (to obtain a flat baseline) and separation. Future work will focus on combining CMCC with MS to screen active compounds from natural products.

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