Progress in cell membrane chromatography

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ABSTRACT: Cell membrane chromatography (CMC) was first established by He *et al.* in 1996. A bioaffinity chromatography technique, CMC has since proven to be an important method for studying drug-receptor interactions and screening active compounds from medicinal herbs. This paper briefly reviews the characteristics of the cell membrane stationary phase (CMSP), the CMC analytical system, and its applications.

Key Words: CMC, preparation, characteristics, applications

Introduction

The receptor concept proposed by Ehrlich and Langley in the early 1900s had little immediate impact upon pharmacology until Clark posited the interaction between the drug and the receptor in 1937 (*1*,*2*). Since that time, drug-receptor interaction has been a major part of receptor pharmacology. Applications of various new techniques in this field, such as the widespread radioligand-binding assay (RBA), have brought about the formation and development of receptor pharmacology (*3*,*4*). However, RBA results have difficulty reflecting the type of force and stereo-selectivity of drug-receptor interactions. There is also limited ability to incorporate radioactive atoms into the structures of most drugs. Thus, direct information on drug-receptor interactions cannot be obtained using the RBA method for drugs that are not radioactively labeled. In 1996 (*5*), the authors developed a new technique for bioaffinity chromatography called cell membrane chromatography (CMC). Since then, it has been used to study drug-receptor interactions and screen for active components from medicinal herbs. In the CMC system, the cell membrane stationary phase (CMSP) was prepared by immobilizing a cell membrane containing special receptors on a silica carrier. Interactions between drugs and receptors have been investigated directly using the CMC system. This system can readily identify active components acting on receptors in the CMSP. The CMC method is considered an important type of bio-membrane chromatography. The following briefly discusses several studies using the CMC method.

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Characteristics of the CMSP

In contrast to normal liquid chromatography, an enzyme-activity-like cell membrane preparation must be maintained in RBA for the CMSP used in CMC. The procedures usually used for preparation of the CMSP and measurement of the surface features of the CMSP are described below (*6*-*8*).

Preparation of the cell membrane

Cells from tissues or cultured cells are dissociated by a hypoosmotic solution and centrifuged to remove nuclei and then centrifuged again to yield the cell membrane. The purity of the cell membrane is verified using a scanning electron microscope. The fact that the flaps of the cytoplasm membrane and the vesicles of the membrane structure can be clearly observed indicates that the procedure for preparing the cell membrane is suitable for use. The total ATPase activity and the protein level of the membrane should be determined when the cell membrane is stored.

Preparation of the CMSP

The key step in CMC is the preparation of the CMSP. Activated silica is placed in a reaction tube, which is followed by suspension of the cell membrane. Adsorption of the cell membrane on the activated silica surface takes place until equilibrium is reached. The whole adsorption process is carried out under vacuum and ultrasonication so that the cell membrane is distributed uniformly on the silica surface. Afterwards, the reaction mixture is diluted with an equal volume of deionized water. The phospholipids of the living cell membrane are able to fuse spontaneously with

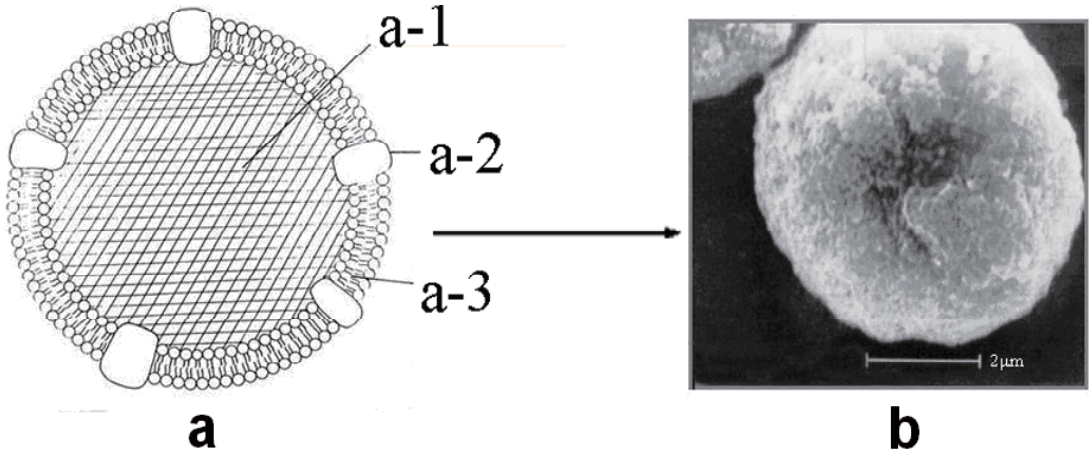

Figure 1. An ideal image and actual micrograph of the CMSP. a: ideal image of the CMSP. a-1, silica carrier; a-2, membrane receptor; a-3, phospholipid layer. b: actual micrograph of the CMSP.

each other (self-fusion) on the silica surface in the aqueous solution until a resealed cell membrane layer is obtained. The supernatant in the reaction mixture is removed by centrifugation and the CMSP is then washed with Tris-HC1 buffer until no residual free cell membrane is detected on its surface. The purity of the cell membrane is verified using a scanning electron microscope.

Surface characteristics of the CMSP

In an aqueous solution, silanol groups (Si-OH) on a silica surface are very strongly polar and usually display strong, irreversible adsorption of biopolymers. Thus, the cell membrane is immobilized on the silica surface. However, one of the characteristics of cell membranes is to have a phospholipid bilayer with two kinds of strong interactions: ionic interaction among membrane polar heads and hydrophobic interaction among carbon chains in the interior of the cell membrane. An ideal image and actual micrograph of the CMSP are shown in Figure 1.

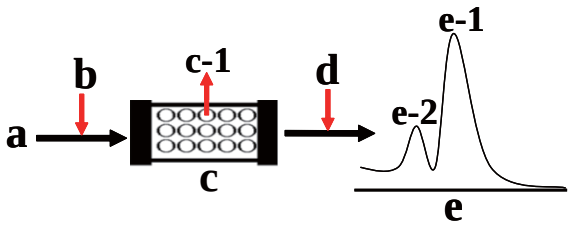

Figure 2. A diagram of the CMC/UV system. a, transfer pump for mobile phase; b, sample; c, CMC column; c-1, CMSP; d, UV-D; e, retention curve; e-1, solvent peak; e-2, retention peak.

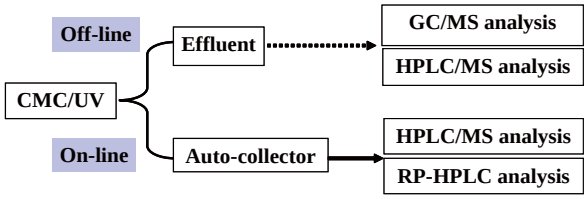

Figure 3. Combined system depicting CMC.

CMC Model System

Chromatographic system

The cell membrane is an important type of biomembrane that has enzymatic activity. In the CMC model system, the chromatographic conditions must imitate the physiological state as much as possible in order to maintain the activity of the CMSP while this system is running. Thus, typical CMC conditions usually include a sodium phosphate buffer or double-distilled water as a mobile phase and a column temperature of 37°C. Additional conditions include a lower flow rate and a detection wavelength suited to detection with a UV detector.

Analytical instruments in CMC

(1) *CMC/UV system*

The CMC/UV system is a general one (Figure 2). It can be used to study drug-receptor interactions. The results obtained from this system correspond well with those of RBA. The system can also be used to screen for active components.

(2) *Combined system*

A combined system can improve CMC qualitatively and quantitatively. This system is well-suited to screening for or identifying active components or compounds from traditional Chinese medicines, natural plants, and reaction mixtures in chemical synthesis through use of either an off-line or an on-line system as shown in Figure 3.

Typical Applications

Drug-receptor interactions

The CMC system has been applied extensively to the study of drug-receptor interactions and measurement of the affinity between drugs and receptors. Yuan and colleagues (*9,10*) used nine ligands of the α_1-adrenergic receptor (AR) to investigate their chromatographic affinity for the α_{1D}-AR subtype. Human embryonic kidney (HEK) 293 cells expressed by cDNA of α_{1D}-AR subtypes were cultured and the CMSP was prepared. Then, the interactions between ligands and α_{1D}-AR in the CMSP were investigated using CMC. Their results showed that the prepared CMSP and CMC method were useful in evaluating affinities of drug-receptor and drug-receptor subtypes and screening for drugs selective to α_{1D}-AR. Yuan and He (*11-13*) prepared a CMSP and used it for rapid on-line chromatographic evaluation of ligand binding affinity to muscarinic acetylcholine receptor (mAChR) by immobilizing the rat cerebral cell membrane and guinea pig jejunum membrane on the surface of a silica carrier. Their data reflected the selectivity and specificity of interactions between drugs and mAChR and proved that CMC can be used to evaluate drug-receptor affinity for drug candidates. At the same time, Yuan and He (*14*) also prepared a CMSP of an expressed cell line and rabbit hepatocytes to study drug-receptor interactions.

In light of these findings, the CMC method can be used to investigate drug-receptor interactions. The results obtained from CMC correspond well with those of RBA.

Screening for active components from medicinal herbs

Medicinal herbs are very important natural resources for finding active compounds as part of new drug development. An effective screening technique is needed for such studies. The CMC method can be used for this purpose because it has both characteristics of chromatographic separation and active recognition from a mixture sample. In actual usage, different CMC models established for several target cells can be used for different medicinal herbs. Several CMC models were used to screen for the effective components from the following natural resources:

(1) *Traditional Chinese medicines*

Traditional Chinese medicines (TCMs) are clinically more effective at treating some diseases. The pharmacological effects of a TCM are usually produced by its active components. Thus, identifying components by means of modern screening techniques is crucial to elucidating the mechanisms and controlling the quality of TCMs. In this area, the CMC model has proven to be a useful screening tool (*15*).

In previous studies, Zhang *et al*. (*16*) and Liang *et al*. (*17*) used a cyno-blood vessel CMC model and rabbit arteriae aorta CMC model in pharmacological trials *in vitro* to screen for effective fractions and effective components of *Angelica sinensis*. They found that the effective fraction was the eluate of hexane-ethyl acetate from the separated extract, and the effective components in the fraction were ligustilide, dimethyl phthalate, and diethyl phthalate, respectively. Li *et al*. (*18*) identified the effective components of *Radix Notoginseng*, *Radix Salviae Miltiorrhizae*, and *Radix Angelicae* with cardiac muscle, cerebrum, and blood vessel CMC models. In accordance with these screening results, a method of controlling the quality of Xinkangping as a TCM prescription for the treatment of coronary heart disease was studied. Zhao *et al*. (*19*) studied the effective components YYH-214 and YYH-216 in the roots and leaves of *herba epimedii* (Yin Yang Huo in Chinese, YYH) screened for using a blood vessel CMC model. They found that YYH-214 and YYH-216 exhibited potent vasodilatation *in vitro*. Screening results provided by the CMC model correlated well with pharmacological effects. Zhang *et al*. (*20*) screened for the effective components of *Cladonia alpestris* (Tai Bai Hua in Chinese, TBH) using a CMC model and studied their correlation with pharmacological effects. They found that TBHG8 was an effective component of TBH1 as an active fraction in TBH for cardiac muscle contractions *in vitro*. Liang *et al*. (*21*) identified the effective components ligustilide and butylidenephthalide from Ligusticum Chuanxiong, used as a traditional Chinese medicine, using a rat artery CMC model. Their results showed that the components effectively inhibited vasoconstriction of rat abdominal aorta segments *in vitro*. These effective components in Ligusticum Chuanxiong are mainly used to treat blood vessel diseases.

(2) *Natural medicinal plants*

In the research and development of new drugs, natural medicinal plants are another important resource in which to search for effective or leading compounds. Using a special target receptor in a CMC model allows the ready identification of bioactive components that react with receptors from natural medicinal plants. Zhang *et al*. (*22*) screened for the active components inhibiting HeLa cell proliferation in *Libanotis buethorimensis* using CMC and found that osthol in *Libanotis buethorimensis* may inhibit HeLa cell proliferation. He *et al*. screened the anti-angiogenesis activity of taspine from *Leontice robustum* using a human umbilical vein endothelial cell (HUVEC) CMC model (*23,24*). Further studies found that taspine may inhibit proliferation and migration of HUVEC and inhibit CAM neovascularisation. These results indicate

that there is a correlation between CMC screening results and a drug's pharmacological effects. In addition, the anti-inflammatory activity of atractylenolide I and atractylenolide III from the rhizomes of *Atractylodes macrocephala Koidz* was screened using a white blood CMC model (*25,26*). Atractylenolide I and atractylenolide III exhibited good anti-inflammatory action in later studies.

In summary, the CMC system provides an analytical method with a high level of performance, selectivity, and efficiency not only for the study of drug-receptor interactions but also for the identification of active compounds from medicinal herbs. The technique behind the system should prove extremely useful in areas like pharmaceutical analysis, receptor pharmacology, and pharmacochemistry.

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