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# Recent advances in cell membrane chromatography for traditional Chinese medicines analysis

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ABSTRACT

Traditional Chinese medicines (TCMs) have been used for preventative care for thousands of years. The active components are the basis for the pharmacodynamics of TCMs, and they can be an important source of lead compounds. As a bioaffinity chromatography technique, cell membrane chromatography (CMC) has been developed for almost 20 years since 1996. It has been proven to be a useful method for studying drug–receptor interactions and screening active components from medicinal herbs. In our review in 2007 (Drug Discov. Ther., 1 (2007) 104–107), the preparation, identification, evaluation, and preliminary applications of CMC stationary phases were presented. In this article, we briefly review some of the latest progress and applications about CMC including instrument development, research on drug–receptor interactions, screening active components from TCMs, and quality control of TCMs.



## 1. Introduction

Traditional Chinese medicines (TCMs) have been used in China for centuries in the prevention and treatment of human diseases. Due to their long history of use and reliable therapeutic efficacy, TCMs are attracting more interest and acquiring acceptance worldwide. The active components are the basis of the pharmacodynamics of TCMs, which can be an important source of lead compounds discovery. Over 100 new products are in clinical development, particularly as anti-cancer and anti-infective drugs [1]. It is widely recognized that screening lead compounds is an attractive and key starting point in drug development programs. The superior the bioactivity of lead compounds screened, the lower the cost of drug development. However, drug discovery from medicinal plants has traditionally been more time-consuming and more complicated than other drug discovery methods [2]. Undoubtedly, screening models as a powerful tool for hunting lead compounds occupy an essential position in the screening programs [3].

Cell membrane chromatography (CMC) was first proposed by He and Gen [4]. As a bioaffinity chromatography technique, CMC has been proven to be an important method for studying drug–receptor interactions and screening active components from medicinal herbs. In the CMC system, cell membrane stationary phase (CMSP) is prepared by immobilizing the cell membrane containing special receptors on a silica carrier. Interactions between drugs and receptors have been investigated directly using the CMC system. In a word, the method proposes a simple and convenient way for studying the interactions of active components in TCMs with membrane receptors *in vitro* and screening active components from complicated TCMs [5]. In our review in 2007, the preparation, identification, evaluation, and preliminary applications of CMSPs were presented [6]. Although CMC has been used for studying drug–receptor interactions and screening active compounds from medicinal herbs for many years, the specificity, sensitivity and selectivity of CMC at that time was very limited. Because there are various receptors on the surface of the cell membrane of animal tissues and common cell lines [7], many non-target receptors are expressed on the cell membrane and there are fewer, uncontrolled receptors.

Entering the 21st century, with the rapid development of life sciences, cell and molecular biology have made considerable progress. The cell lines with high expression of specific receptors can be constructed. Thus, the CMSP has been improved by using cell lines with high expression of specific receptors. At the same time, HPLC–MS and GC–MS were combined with the CMC system for identification of the screened active components. In this article, some new advances about CMC are reviewed briefly.

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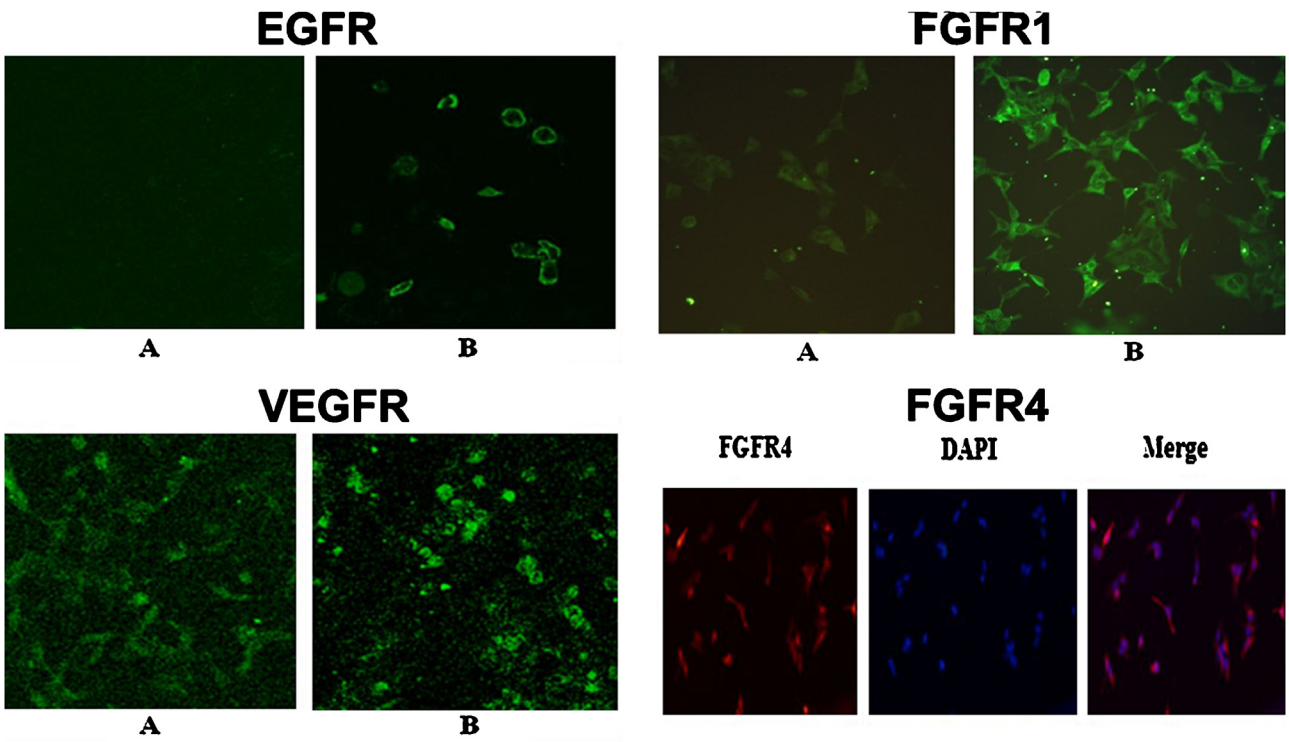


**Fig. 1.** Immunofluorescence figures of four high expression RTK cells. (A) HEK293 cell line; (B) cell lines with high expression of specific receptors. Reprinted from [10], copyright (2010), with permission from Elsevier.

## 2. Construction of target cell line with high receptor expression

Some types of CMSPs with high expression of specific receptors have been prepared and the corresponding CMC models have been developed. As shown in Fig. 1, cells with high expression of epidermal growth factor receptor (EGFR) (HEK293/EGFR) [8], vascular endothelial growth factor receptor (VEGFR-2) (HEK293/VEGFR-2) [9], fibroblast growth factor receptor (FGFR-1) (HEK293/FGFR-1) [10] and FGFR-4 (HEK293/FGFR-4) [11] have been constructed through plasmid construction and stable transfection procedures. The expression of specific receptors in recombinant cell lines is 4–18 times higher than that of the HEK293 cell line. Cells with high expression of $\beta_1$ adrenergic receptor (AR) [12], $\alpha_{1A}$ AR [13] and $\alpha_{1D}$ AR [14] have also been used for screening active components in TCMs. These CMSPs with high expression of receptors have the following characteristics: (1) expression of specific receptors is increased and controllable; (2) uniformity of CMSP surface is enhanced; (3) specificity and selectivity of the CMC method is improved; and (4) sensitivity for identifying trace components is improved.

## 3. Development of CMC instruments

HPLC–MS and GC–MS have been widely used in pharmaceutical, toxicological, environmental and food analysis because of their advantages of high sensitivity and accuracy. Based on the previous CMC method, HPLC–MS and GC–MS have been combined with CMC. The novel combined methods have been developed with the simultaneous functions of active screening and target identification, which have greatly improved the efficiency of drug screening by narrowing the gap from blind to clear for defining active components.

### 3.1. CMC-HPLC–MS

Fig. 2 shows that the CMC system was online-coupled with the HPLC–MS system through a 10-port two-position valve and two ODS C18 enrichment columns (EC1 and EC2, 4.6 × 10 mm, 5 μm). At the beginning (Position A), columns in both dimensions were equilibrated. After a sample was injected into the CMC system, the

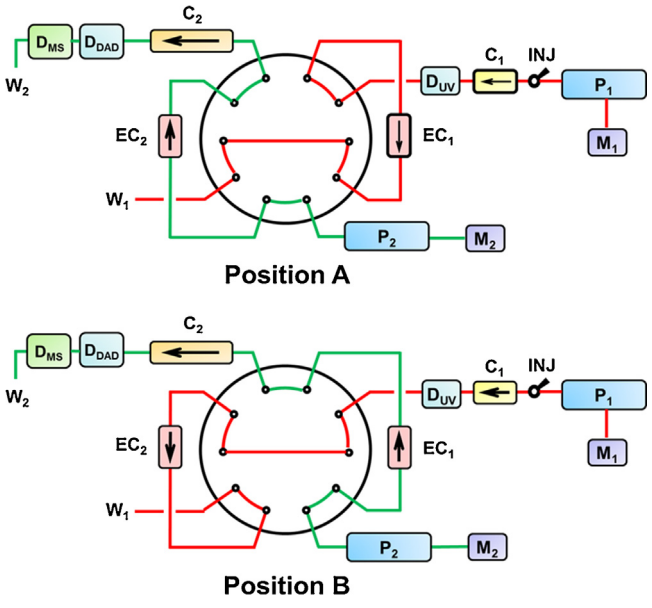


**Fig. 2.** Schematic diagram of CMC-online-HPLC–MS system. UV, ultraviolet detector; DAD/MS, diode array detector/mass spectrometer detector; $C_1$: CMC column. $C_2$: the VP-ODS $C_{18}$ column.
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