

Preparation and Application of Modified VEGFR-2 Cell Membrane Chromatographic Separation System

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Abstract The vascular endothelial growth factor receptor-2 (VEGFR-2) in some tumor cells is a significant target for drug discovery. In this work, a modified model of VEGFR-2 cell membrane stationary phase (CMSP) was prepared by immobilizing U251 cell membrane onto the surface of chitosan-silica (CTS-SiO₂) hybrid carrier. The surface and chromatographic characteristics of VEGFR-2 CMSP were studied. We have developed modified VEGFR-2 cell membrane chromatography for screening drugs and sunitinib malate was used as a positive control. The interaction between the new compounds and membrane receptor was determined by the capacity factors (k'). The *in vitro* cytotoxicity of 10 new compounds on U251 cell viability was determined by MTT test separately to verify the potential pharmacological activity. The modified VEGFR-2 cell membrane chromatographic system demonstrated fast and effective characteristics for screening leading compounds.

Keywords VEGFR-2 cell membrane chromatography · Chitosan-silica · High performance liquid chromatography · Screening new compounds · Sunitinib

Introduction

Vascular endothelial growth factor (VEGF) signaling often represents a critical rate-limiting step in physiological angiogenesis. The VEGFs exert their biologic effect through interaction with their receptors [1]. VEGFR-2 (also known as KDR) is a subtype of VEGFR, and VEGFR-2 expression is restricted primarily to the vasculature and is the key mediator of VEGF-mediated mitogenic effect [2, 3]. VEGFR-2 is expressed in most adult endothelial cells and on circulating endothelial progenitor cells [4]. Abnormal expression of VEGFR-2 leads to several disorders including cancer. As a significant receptor contributing to the progress of tumor, VEGFR-2 has been currently an important target for anti-tumor therapeutics [5, 6]. One of the approaches targeting VEGF/VEGFR axis as anti-cancer therapies is blockage of VEGFR kinase activity with small molecule inhibitors. Many known EGFR agonists including sunitinib, cetuximab, gefitinib and the others are selective for VEGFR. Therefore, they are often used as positive controls [7]. Ten new compounds of anthranilic diamides which are small molecule VEGFR-2 inhibitors in this study were synthesized by computer aided design and different cheminformatics approaches like target identification, active site prediction.

In order to improve the maneuver of drug-receptor interaction research and existed drug screening strategy aiming at a specific receptor, some biological affinity chromatographic systems with better specification on methods have been developed [8]. The incorporation of isolated cytosolic proteins and enzymes into an affinity chromatography column and the application of the resulting column to the study of ligand–protein interactions were first demonstrated by Chaiken and Carr and have been extensively studied [9]. Cell Membrane Chromatography (CMC),

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originated by He et al. according to receptor pharmacology and improved by many groups, has been verified to be an effective chromatographic technique for the study of screening leading compounds acting on a specific receptor [10, 11]. The principle of this model is that the membrane receptors were prepared as cell membrane stationary phase (CMSP). The CMSP was prepared by immobilizing cell membrane on the surface of the carrier and was used for the rapid on-line chromatography. In this system, many kinds of interactions, such as ionic linkages, hydrogen bonds, and hydrophobic bonds between the drug and CMSP mainly contribute to drug retention. Different affinity between drug and cell membranes showed different drug retention. The stronger the affinity is, the longer the drug retention time is. Therefore, the characteristics of drug-receptor interactions can be shown by the capacity factors (k') [10, 11]. In past decades, an enormous number of possible drug candidates have been screened by this technology, some of which have even been applied into clinical research stages [12–16]. Silica as the cell membrane support was proved to be less biocompatible to cell membrane or proteins than some natural polysaccharide (chitosan/sodium alginate) modified silica [17].

The VEGFR-CMC systems have been used for screening bioactive compounds from medicinal plants such as *Aconitum carmichaeli* Debx. [14] and *Ligusticum chuanxiong* [21]. Screening of active components from traditional Chinese herb *Aconitum carmichaeli* Debx. acting on VEGFR-2 has been completed by using an online coupled CMC with LC/MS [14]. The CMC stationary phase was prepared by loading the cell membrane of HEK293/VEGFR onto the activated silica. Three bioactive components were identified from *Aconitum carmichaeli* Debx. [14]. The VEGFR-CMC system for screening bioactive compounds from *Ligusticum chuanxiong* was prepared by attaching cells onto amino microspheres using HUVECs as a probe [21]. The high stability of this cell membrane-based stationary phase was due to the high affinity between integrin on the cells and RGD peptide that was coated onto the poly[oligo (ethylene glycol) methacrylate] material [21].

In this work, we used a modified CMC system loaded with the cell membrane of U251/VEGFR-2 as the stationary phase in a VEGFR-CMC model to screen 10 newly synthesized compounds of anthranilic diamides in order to determine their anti-tumor activities. The chitosan/silica hybrid stationary phase material developed had better adsorption ability and biocompatibility than pure silica. The method was relative low-cost in the long term. Moreover, chitosan is nontoxic, environment-friendly and has many significant biological and chemical properties such as biocompatibility, bioactivity and biodegradability [17].

Experimental

Chemicals and Reagents

Silica gel (5 μm , 200 \AA) was purchased from Qingdao Meigao Chemical Co., Ltd. (Qingdao, China). Sunitinib malate was from Nanjing Ange Pharmaceutical Co., Ltd. (Nanjing, China). HPLC grade methanol was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Chitosan was purchased from Shanghai Kayon Biological Technology Co., Ltd. (Shanghai, China). Epoxy chloropropane, sodium hydroxide, isopropanol and glacial acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). DMEM was obtained from Invitrogen Corporation (Grand Island, USA). A human umbilical vein endothelial cells (HUVEC-2) derived from Cell application, Inc. U251 was purchased from Shanghai Institutes of Cell Biology in the Chinese Academy of Sciences (Shanghai, China). Hela, PC-3, MCF-7, A549 cells were supplied by the Institute of Immunopharmacology and Immunotherapy, School of Pharmaceutical Sciences, Shandong University, Jinan, China. Fetal bovine serum was purchased from Hangzhou Sijiqing Serum Works. Trypsin/EDTA (Sigma, Germany), PE mouse anti-human CD309 (VEGFR-2) (No. 560872) and PE Mouse IgG1, κ Isotype Control (No. 554680) were purchased from BD Pharmingen, USA. New compounds were synthesized by Medicinal Chemistry Lab of School of Pharmaceutical Sciences, Shandong University. Water was of HPLC grade. All other reagents and solvents were of analytical reagent grade and were used without further purification unless noted otherwise.

Apparatus and Conditions

CMC analysis was performed on an Agilent 1260 instrument that consisted of G1311B Quat Pump, G1367E Hip ALS, G1316A Column Oven, and G4212A Diode Array Detector (Agilent, USA). The data were acquired using Agilent chromatographic working station. Model 680 microplate reader was from Bio-Rad Laboratories, Inc. (CA, USA). Scanning electron microscopic analysis was performed using JSM-6700F scanning electron microscope (JEOL, Japan). FTIR spectra were produced using NEXUS 470 Fourier transform infrared spectrometer (Nicolet, USA). Thermogravimetric data were obtained using TGA/DSC1/1600HT thermo gravimetric analyzer (Mettler, Swiss). Elemental results were generated using Vario EL3 elemental analyzer (Elementar, Germany). Flow cytometric analysis was done using FACS Calibur flow cytometer (BD Bioscience). High-speed refrigerated centrifuge was the product of Eppendorf (5810R, Eppendorf, Germany).

The CMC mobile phase consisted of phosphate-buffered saline (25 mM PBS, pH 7.4) and was delivered at a flow

rate of 0.2 mL/min. All measurements were performed with a diode array detector and column temperature was set at 37 ± 0.5 °C.

Preparation of Cell Membrane Supports

The CTS/SiO₂ hybrid material was prepared with some modifications as described by Zhang et al. In brief, 0.78 g silica was dispersed in 16 mL acetone, and then 200 µL epoxy chloropropane was added. The mixture was heated to reflux for 8 h and cooled at room temperature. The residual solvent and epoxy chloropropane were then evaporated under reduced pressure. The powder was dried at 60 °C for 3 h in vacuum and the hydroxypropyl-chlorine silica gel was obtained.

To the hydroxypropyl-chlorine SiO₂ (0.6 g), 0.5 g chitosan, 0.08 g sodium hydroxide and 12 mL isopropanol were added in a flask and heated to 70 °C, and then the mixture reacted for 4 h under constant stirring. After the mixture was washed with isopropanol and water for several times, it was dried at 80 °C for 5 h in vacuum. Finally, the mixture was dialyzed against deionized water to remove sodium chloride. In order to remove residual chitosan, the mixture was incubated in 1 M acetic acid solution at room temperature for 2 h, and then the beads were washed with deionized water to neutral. The powder was dried at 80 °C for 12 h.

Characterization of CTS/SiO₂ Hybrid Material

The CTS-SiO₂ hybrid material was characterized by means of scanning electron microscope (SEM), Fourier transform infrared (FTIR), thermogravimetric and elemental analyses. The SEM images of SiO₂ and CTS-SiO₂ hybrid materials were taken using a scanning electron microscope. Samples were coated with an approximate film of gold, and the SEM images were taken at an accelerating voltage of 26 kV. The FTIR spectra of the pellets were recorded in absorption mode in the range of 4000–400 cm⁻¹. The samples were blended with KBr pellets. Thermal stability of the hybrid membranes was analyzed in N₂ atmosphere at the speed of 20 mL min⁻¹. The temperature was ranged from 30 to 600 °C at a scanning rate of 15 °C min⁻¹. To verify whether chitosan was bonded with SiO₂, carbon, hydrogen and nitrogen contents were quantified by the elemental analyzer. Immobilized gelatinase method was used to assess biocompatibility of CTS-SiO₂ hybrid and pure SiO₂ materials, and the enzyme characteristic parameters (K_m) were then calculated.

Cells Expressing VEGFR-2 Screening by Flow Cytometry Analysis

Flow cytometry assay was conducted to provide information on VEGFR-2 expression on the surface of cells. All

cells (U251, Hela, PC-3, MCF-7, A549 cell lines) were detached from the bottom of a flask by incubation with trypsin/EDTA solution at room temperature, washed with cold PBS and stained with PE-conjugated mouse IgG1 κ isotype control or a PE-conjugated mouse anti-human CD309 (VEGFR-2). Finally, the cells were resuspended in 200 µL PBS and at least 10000 cells were examined for each cell population. To compare the surface densities of VEGFR-2 on all kinds of cells, we calculated the expression levels of cells stained with PE-conjugated mouse anti-human CD309 (VEGFR-2) and isotype control. Cells were acquired using a FACS Calibur flow cytometer (BD Bioscience) and analyzed using WinMDI 2.9 software.

Optimization of Cell Disruption

Five kinds of methods were optimized to achieve proper cell disruption, respectively. In the first method, cells were disrupted by the ultrasonic cleaner for 30 min at the power of 100 W. The manual homogenizer was used in the second method to disrupte cells for 10 min. The third used the combination of manual homogenization and ultrasonication. The automatic homogenizer was used in the fourth method for the disruption of 6 min. In the last method, the cells were ultrasonicated for 2 s at the power of 400 W by the ultrasonic cell disruptor. The resulting suspension was detected by the reverse microscope to make sure their complete disruption. Then, the concentration of membrane protein and enzymatic activity were determined in the suspensions.

Cell Membrane Stationary Phase

The U251 cells (8×10^6) were washed twice with PBS by centrifuging at $110 \times g$ for 10 min at 4 °C. Tris-HCl (50 mM, pH 7.4) was added to produce cell suspension, which was ruptured by the supersonic procedure for 4 s under iced bath. The resulting homogenate was centrifuged at $1000 \times g$ for 10 min. The pellet was discarded and the supernatant was centrifuged at $14000 \times g$ for 20 min at 4 °C. The precipitation was suspended in 8 mL Tris-HCl (50 mM, pH 7.4), and the suspension was centrifuged at $14000 \times g$ again. U251 cell membrane suspension in 5 mL distilled water was obtained. According to the literatures, CMSP was prepared by the adsorption of cell membrane suspension (5 mL) on the chitosan-silica (0.04 g) under the vacuum and agitation conditions at 4 °C. The VEGFR-2 CMSP was packed into the column (10×2.0 mm I.D.) using a wet packing procedure.

Validation Application of VEGFR-CMC Module

Specificity of the VEGFR-CMC systems was investigated. Sunitinib, small molecular receptor tyrosine kinase

inhibitor, has been approved by Food and Drug Administration as the first-line therapy for the carcinoma. This inhibitor with the antiangiogenic activity via targeting the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) was used as the pharmacological agent in many studies [18]. U251 cell lines were found to have high abundance of receptor VEGFR-2, so sunitinib U251 cell lines were selected to validate the specificity of the model system established. The retention times on the CTS/SiO₂ cell membrane and CTS/SiO₂ chromatographic columns were determined to confirm whether the cell membrane was combined with sunitinib specifically. The content of the membrane protein with pre-column and post-column after usage was identified to investigate the stability. To improve peak shape and retention time, the mobile phase was also optimized.

Sample Preparation

The standard stock solution of sunitinib (5 mM) was prepared in methanol. New compounds solutions of A1, A3, A4, B1, B3, B4, C1, C2, C3 and C4 (5 mM each) were also prepared in methanol. The working solutions (0.2 mM) were diluted with methanol. All solvents were filtered through a 0.45 μm membrane filter.

Application

This VEGFR-CMC system was applied for screening VEGFR-2 antagonists using sunitinib as a control drug. The standard solution and newly synthesized compounds were analyzed. After the VEGFR-2/CMC system reached the equilibrium, 10 μL sunitinib and new compounds were injected into the system and the capacity factors (k') of sunitinib and new compounds were determined using the equation below.

$$k' = (t_R - t_0)/t_0$$

where t_R and t_0 are the retention times of the solute and no-retention solvent, respectively [19].

Cell Viability Assay

The effect of new compounds on cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, exponentially growing U251 cells were harvested and plated in 96-well plates at a concentration of 5×10^3 cells/well. After 24 h incubation at 37 °C, cells were treated with new compounds at various concentrations for 72 h. Then, 20 μL of MTT (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. After the supernatant was discarded, 150 μL

of DMSO was added to each well, and the optical density of cells was determined with a microplate reader (Bio-Rad Instruments, USA) at 490 nm and expressed as absorbance values [20].

Results and Discussion

Characterization of CTS/SiO₂ Hybrid Material

As shown in Fig. 1, the surface of the pure SiO₂ was found to be relatively smooth and homogenous (Fig. 1a), which was significantly different from the CTS/SiO₂ hybrid material. It was also obvious that the CTS/SiO₂ hybrid material exhibited a rough and irregular surface morphology (Fig. 1b). The more uniform space between the CTS/SiO₂ hybrid material was suitable for the preparation of the stationary phase. The FTIR spectra of the pure SiO₂, CTS/SiO₂ hybrid material and chitosan are shown in Fig. 1c. As can be seen, the spectrum of the CTS/SiO₂ hybrid material exhibited characteristic peaks of both SiO₂ and chitosan, indicating that chitosan was bonded successfully with SiO₂ to form the hybrid material. In the TGA and DSC analysis (Fig. 1d), the curves of pure SiO₂, CTS-SiO₂ hybrid material and chitosan exhibited that the physical desorption of bound water occurred below 120 °C and the rapid degradation at more than 260 °C was caused by chitosan chain decomposition and oxidation degradation. The formation of CTS/SiO₂ hybrid material weakened the hydrogen bonding forces between the molecules in the chain of chitosan, which decreased the thermal decomposition temperature of chitosan. DSC curves showed that thermal decomposition temperature of CTS/SiO₂ hybrid material was different from that of the physical mixtures, which indicated the generation of new chemical bonds. The elemental analysis results (Table 1) illustrate that the CTS/SiO₂ hybrid material was synthesized successfully. The gelatinase immobilized quantity and K_m of silica and CTS/SiO₂ hybrid materials are shown in Table 2. The immobilized enzyme amounts and K_m of the CTS/SiO₂ material were more desirable than those of the silica material, suggesting better combination between the CTS/SiO₂ hybrid material and cell membranes.

Cell Screening and Optimization of Cell Disruption and Centrifugal Force

As shown in Fig. 2, the results analyzed by flow cytometry indicate that U251 expressed the highest level of VEGFR-2 in the tumor cells. The cell membrane

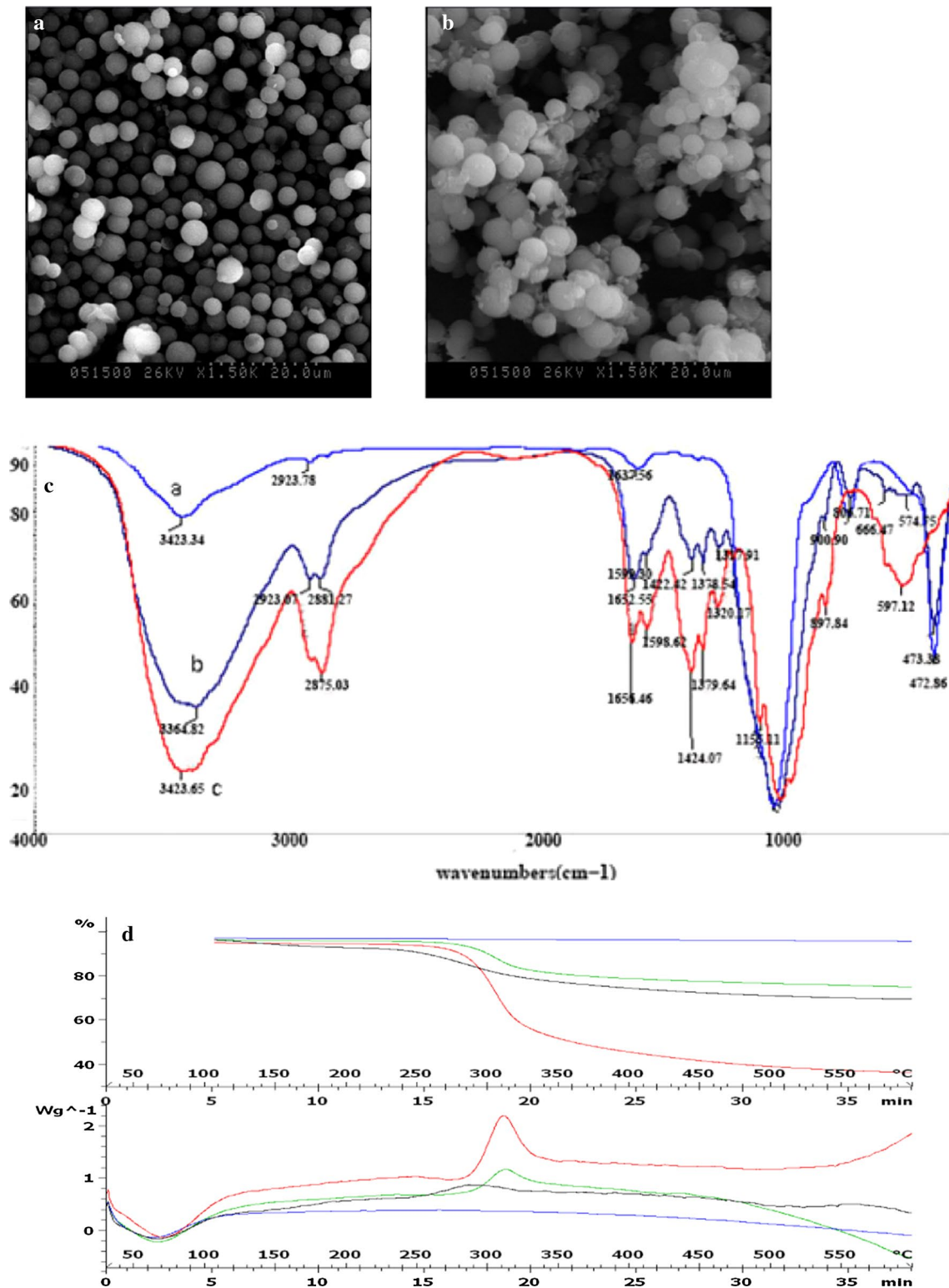


Fig. 1 Characterization of CTS/SiO₂ hybrid material. **a** Scanning electron micrograph of pure SiO₂ ($\times 1500$); **b** scanning electron micrograph of CTS/SiO₂ hybrid material ($\times 1500$). **c** FTIR spectra of different materials; **a**: SiO₂; **b**: CTS/SiO₂ hybrid material; **c**: chitosan.

d TGA and DSC results of different materials. *Red curve* chitosan; *green curve* physical mixture of chitosan and SiO₂; *blue curve*: SiO₂; *black curve*: CTS/SiO₂ hybrid material

Table 1 The results of elemental analysis

CTS/SiO ₂ material	Weight (mg)	C		H		N	
		Content (%)	Peak area	Content (%)	Peak area	Content (%)	Peak area
1	2.6220	16.66	11797	3.222	6969	2.327	2321
2	2.6190	16.57	11717	3.333	7222	2.324	2315
Average	2.6205	16.62	11757	3.278	7096	2.326	2318

Table 2 The contents and K_m's of immobilized gelatinases

	Content (U/g)	K _m (mmol/L)
Free enzymes	–	0.013 ± 0.0011
SiO ₂	4968 ± 143	0.33 ± 0.0015
CTS-SiO ₂	5262 ± 120	0.075 ± 0.0018

Data was present as mean ± SD (*n* = 3)

chromatography experiment needed a large amount of cells, so we did not select the HUVEC due to its slow growth. The comparison data among different assays are shown in Fig. 3. The ultrasonicated disruption was chosen

because of higher membrane proteins and enzymatic activity.

The Surface Characteristic of VEGFR-2 CMSP

The results shown in Fig. 4a, b indicate that the surface of chitosan-silica carrier was completely covered and integrated with U251 cell membrane and the surface morphology of U251 CMSP was very different from that of pure chitosan-silica carrier. As shown in Fig. 5, sunitinib had stronger retention onto the CTS/SiO₂ cell membrane chromatographic column than the CTS/SiO₂ chromatographic column. These results indicate that sunitinib was strongly associated with VEGFR-2 cell membrane and U251 CMSP

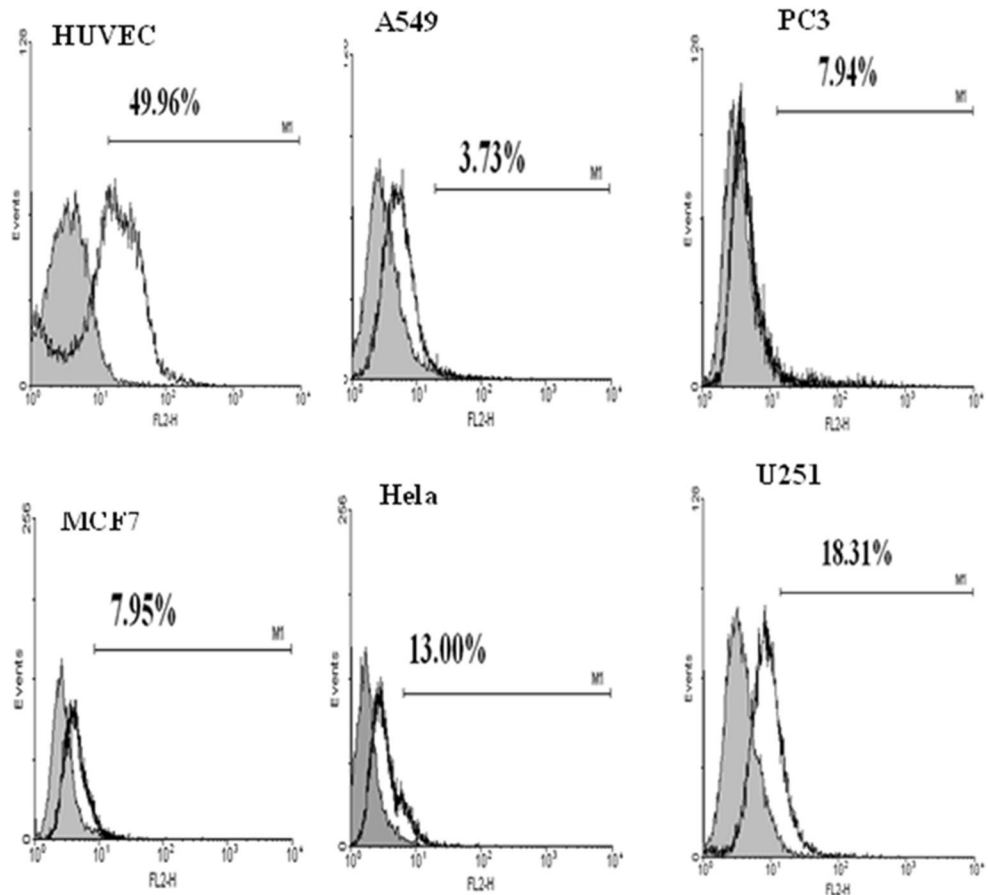
Fig. 2 The expressions of VEGFR-2 on different cells that were analyzed by flow cytometry

Fig. 3 The contents and enzymatic activities of membrane proteins that were produced by 5 different disruption methods. The conditions for 5 different disruption methods: the cells were disrupted by (1) the ultrasonic cleaner for 30 min at the power of 100 W, (2) the manual homogenizer disrupted cells for 10 min, (3) both the manual homogenizer and ultrasonic cleaner, (4) the automatic homogenizer for 6 min (5) the ultrasonic cell disruptor for 2 s at the power of 400 W

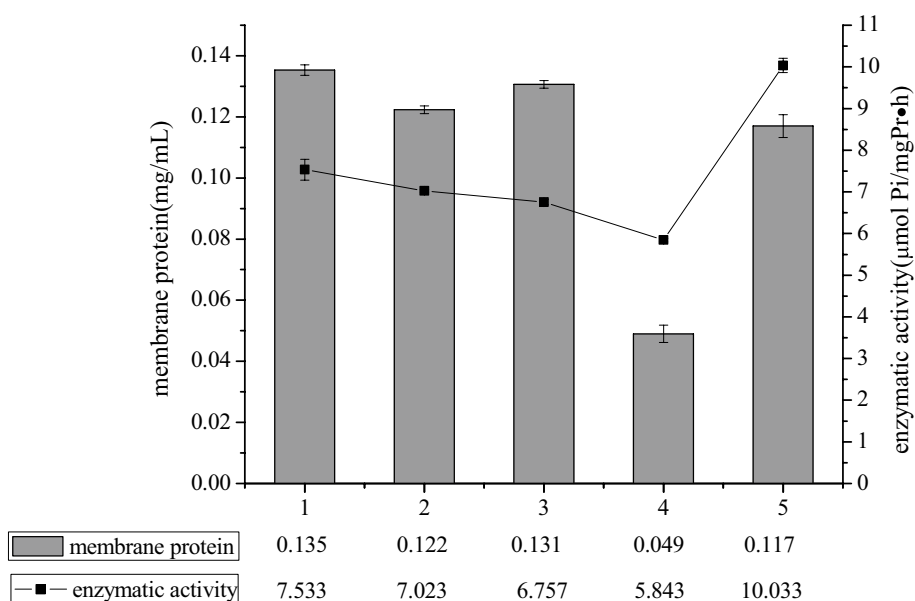
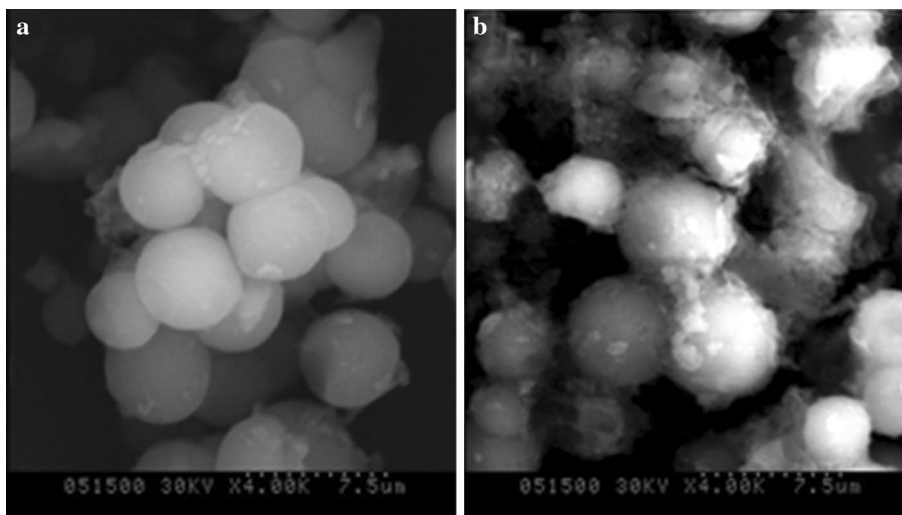


Fig. 4 The surface characteristics of VEGFR-2 CMSP cell membrane stationary phase. The scanning electron micrographs: **a** pure chitosan-silica carrier and **b** U251 cell membrane stationary phase; the magnification for both micrographs was 4000



system was prepared satisfactorily. The stability of both CTS/SiO₂ CMC and SiO₂ CMC columns was satisfactory for at least 1 day based on the t test since the column pressure had a dramatic increase approximately from 10 to 100 bar when the SiO₂ CMC column was used in the following day. By contrast, the life span of the CTS/SiO₂ CMC column was much longer because no column pressure increase was observed even after 5 days. In addition, the protein content was superior for the CTS/SiO₂ CMC column after 1 day's usage (Table 3). When the flow rate was set at 0.2 mL/min, the retention time and the peak shape of the positive control drug were the most favorable when the 25 mM PBS was used as the mobile phase (Fig. 6).

Application of VEGFR-CMC System

The capacity factor k' is specific for a given substance and can reflect the partition between stationary and mobile phases at equilibrium. It depends on the stationary phase, the mobile phase, the temperature, the quality of the packing, and so on. In the CMC experiment, because the chitosan-silica surface was coated by U251 cell membrane in CMSP, k' was used to describe the ligands-CMSP interactions. The capacity factor k' rank order measured from VEGFR-2 cell membrane chromatographic column for 10 new compounds and sunitinib was sunitinib>B4>A4>B3>A3>C4>C3>B1>C2>C1/A1 (Fig. 7). The interactions between C1/A1 and cell membrane was so weak that they

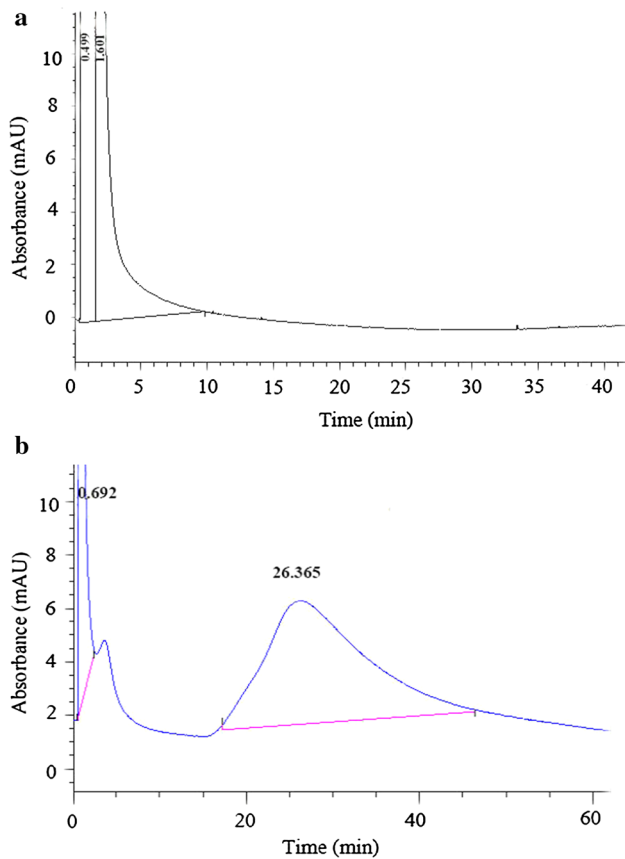


Fig. 5 HPLC chromatograms of sunitinib on **a** CTS/SiO₂ column and **b** CTS/SiO₂ cell membrane column. The chromatographic conditions: column dimension: 10 mm × 2.0 mm; mobile phase: phosphate-buffered saline solution (25 mM, pH 7.4); detection wavelength: 262 nm, column temperature: 37 °C

came out in the solvent front ($t_R = t_0$). In parallel experiments using pure chitosan-silica stationary phase (the negative control), retention time of test compounds was less than 2 min and no specific binding was detected.

Cell Viability Assay

The interaction between compounds and a specific receptor is the premise of being a drug. But not every compound which interacts with cell membrane receptor could show a satisfactory effect in the pharmacological activity. MTT

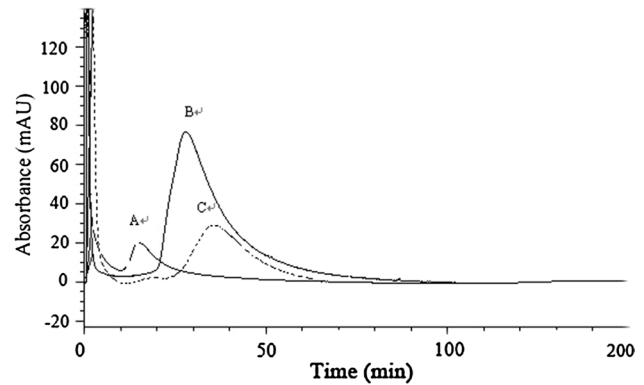


Fig. 6 The elution chromatograms of sunitinib under the different mobile phase conditions. The chromatographic conditions: mobile phases: **a** 50 mol/L PBS solution (pH 7.4); **b** 25 mol/L PBS solution (pH 7.4); **c** 12.5 mol/L PBS solution (pH 7.4); column: CTS/SiO₂ cell membrane column, 10 mm × 2.0 mm; wavelength: 262 nm; column temperature: 37 °C

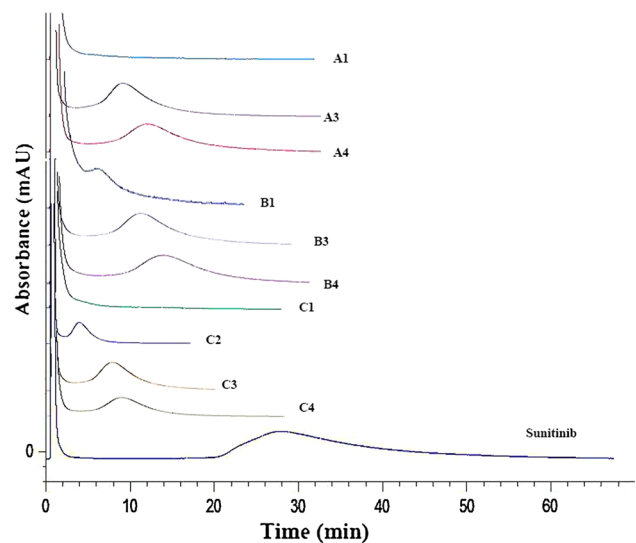


Fig. 7 Elution profiles of 10 newly synthesized compounds and sunitinib on the VEGFR-2 cell membrane chromatographic column. The chromatographic conditions: column: CTS/SiO₂ cell membrane column, 10 mm × 2.0 mm; mobile phase: PBS solution, 25 mM, pH 7.4; wavelength: 262 nm; column temperature: 37 °C

assay is usually used to show the cytotoxicity of compounds on cells. In this work, the VEGFR-2/CMC system that used VEGFR-2 cell membrane as stationary phase could

Table 3 The contents of the membrane protein with pre-column and post-column after usage

	1 day after usage of SiO ₂ column	1 day after usage of CTS/SiO ₂ column	5 day after usage of SiO ₂ column
Pre-column (mg/g)	10.102 ± 3.0	13.351 ± 6.2	9.00 ± 2.8
Post-column (mg/g)	10.148 ± 3.8	13.665 ± 4.9	9.03 ± 3.5

Data was present as mean ± SD ($n = 3$)

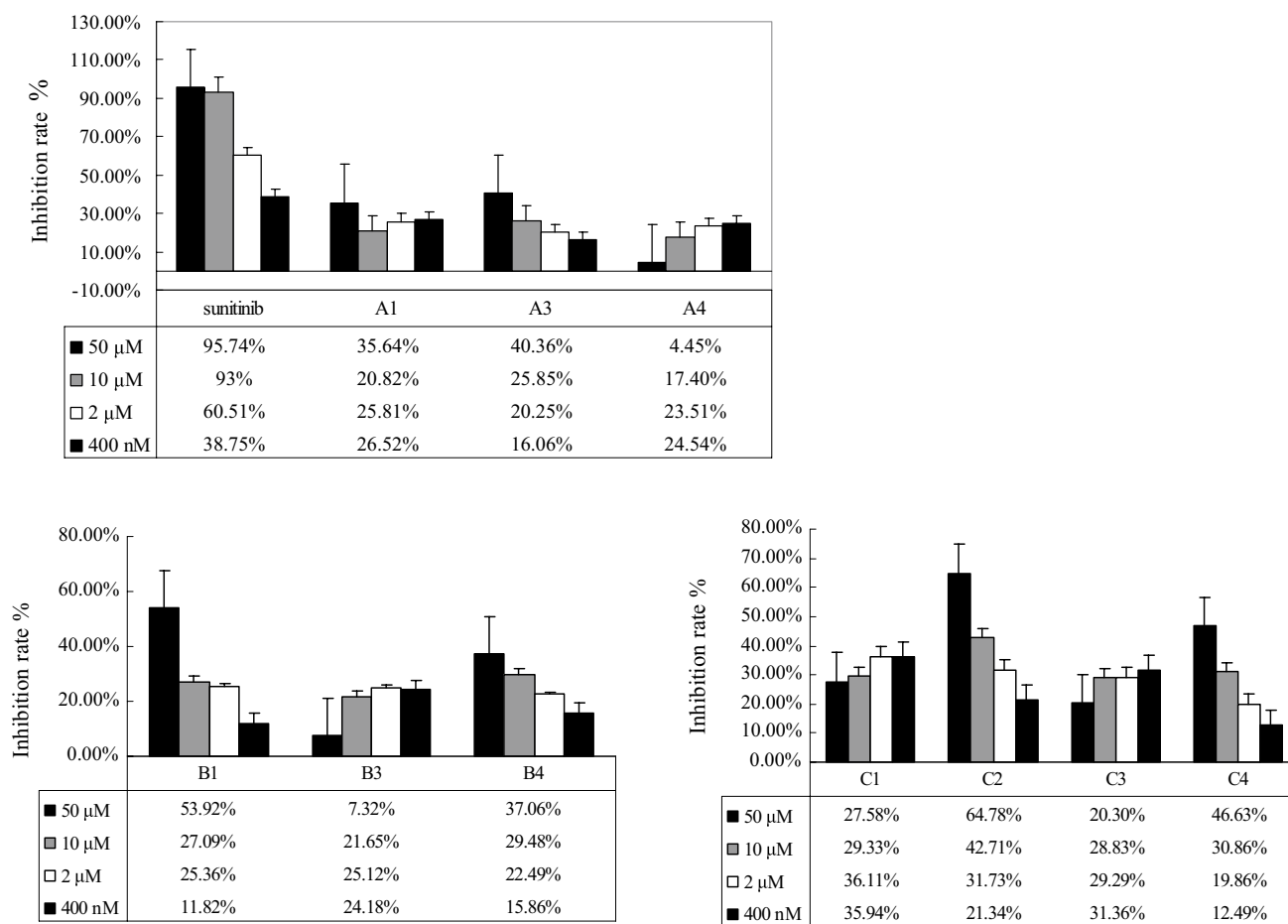


Fig. 8 Inhibition rates of sunitinib and newly synthesized compounds on U251 cell

selectively recognize VEGFR-2 antagonists such as sunitinib. Sunitinib was also used as a control drug for measuring the biological activity. As depicted in Fig. 8, sunitinib showed obvious and dose-dependent inhibition activity in the dose range of 0.4–50 μM . The inhibition activity of 10 new

compounds was found to be weaker than that of sunitinib, and it also depended on doses.

One of the aims for establishing the CMC model in this work was to study the correlation between the chromatographic parameters and pharmacological action. Basing on the

Table 4 Retentions and capacity factors of sunitinib and 10 new compounds on VEGFR-2 CTS/SiO₂ CMSP

Compounds	t_0 Mean (min)	t_R Mean (min)	k'	RSD (%)
Sunitinib	0.672	26.772 \pm 0.187	38.84 \pm 0.27	0.70
A1	0.687	–	–	–
A3	0.682	6.171 \pm 0.010	8.05 \pm 0.01	0.15
A4	0.745	7.471 \pm 0.102	9.03 \pm 0.12	1.37
B1	0.668	4.372 \pm 0.074	5.54 \pm 0.09	1.69
B3	0.680	6.763 \pm 0.015	8.95 \pm 0.02	0.22
B4	0.713	7.964 \pm 0.062	10.17 \pm 0.08	0.78
C1	0.680	–	–	–
C2	0.730	2.125 \pm 0.007	1.91 \pm 0.01	0.27
C3	0.711	4.752 \pm 0.011	5.68 \pm 0.01	0.23
C4	0.701	5.181 \pm 0.054	6.39 \pm 0.07	1.04

capacity factor (k') values of sunitinib and new compounds, the following conclusions can be established: (1) the strength of interaction of sunitinib was much greater than that of new compounds on the VEGFR-2 CMSP. (2) The 10 new compounds had various interaction strengths on the VEGFR-2 CMSP as shown in Table 4 and Fig. 7. These results indicate that the chromatographic parameters of VEGFR-2 inhibitors acting on the VEGFR-2 CMSP were found to have a very good correlation with their pharmacological actions. The results in this study indicate that the VEGFR-2/CMC system could be used directly to imitate the interaction process between a drug and the target (membrane receptor) in vivo.

Conclusion

The modified VEGFR-2/CMC system based on the chitosan/silica hybrid base material developed in this work increased the selectivity and sensitivity of bioactive compound screening and improved the enzymatic activity and stability of CMC stationary phase. The cytotoxicity of these new compounds determined on the online modified VEGFR-2/CMC system was confirmed by the conventional MTT assay, which further verified these compounds' bioactivity and supported the separation abilities of this model. The CMC model developed in this work could simulate the interaction between drugs and membrane and its receptor in vitro and could be used to study the leading compounds in drug discovery for the fast and effective initial screening system.

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Compliance with Ethical Standards

Conflict of interest All author declares that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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