

# Simulation Design of Exosomes Separation Microfluid Device Based on Asymmetrical Flow Field-Flow Fractionation



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**Abstract** Exosomes are extracellular nano-scale vesicles with a diameter of about 40–100 nm and a density of about 1.13–1.19 g/ml, which are secreted by most cells and have phospholipid bilayer structure on their surface. Exosomes contain a large amount of genetic information and a variety of proteins involved in cell–cell communication and macromolecular transmission. At present, the importance of exosomes in the early diagnosis and targeted treatment of diseases is widely noticed. Thus, efficient separation method for exosomes separate is urgent. In this paper, the flow field-flow fractionation method was used to separate the latter from the mixture of BSA and exosomes. Model construction and simulation were performed using commercial software COMSOL Multiphysics. According to the results of the simulation and theoretical analysis, the method can achieve rapid, effective and compatible separation of exosomes. Finally, according to the simulation results, some reference opinions can be provided for the actual microfluidic chip production, and the subsequent applications are prospected.

**Keywords** Exosomes · Flow field-flow fractionation · Separation · Simulation · Microfluidic

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## 1 Introduction

Exosomes have been the focus of researchers since their discovery and naming in 1987 [1]. Nowadays, exosomes have great potential as circulating biomarkers of disease, and it can also be used as a medium for remote intercellular communication in the body. Biofluids (such as blood, saliva, urine, etc.) contain a large number of exosomes that can transfer various molecules from parental cells to other cells, including proteins, mRNA/miRNA and DNA, so it can be used as a cell replacement [2]. In addition, exosomes are also a significant advantage as cancer monitors [3].

Exosomes are relatively new targets for bioassays that have unique physical and biological properties: Their size range (typically 40–100 nm in diameter) is much smaller than cells (10–30  $\mu\text{m}$ ), but larger than protein and comprises a highly heterogeneous constituency [4]. Traditional methods for separating exosomes are as follows: ultracentrifugation, sucrose-gradient centrifugation, size-exclusion chromatography [5], etc. They all have some limitations, such as time-consuming, low recovery rate or low purity. Thus, efficient separation method for exosomes separate is urgent.

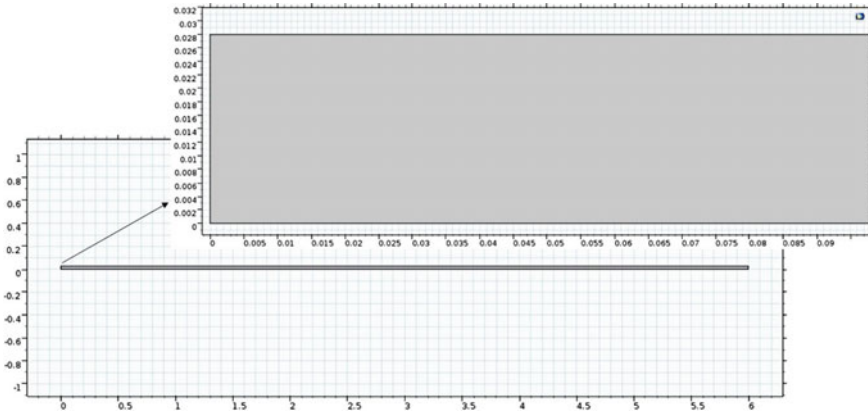
Asymmetrical flow field-flow fractionation (AF4) is a sub-class of field-flow fractionation techniques developed by Giddings in 1966 [6]. It is based on the difference in diffusion coefficient to complete the sample separation. Separation is done in a flat channel, the channels are distributed in a laminar flow, and in the direction of the vertical laminar flow, another flow field is applied, which is called a cross-flow. Samples with different diffusion coefficients are balanced at different locations in the channel by cross-flow to move toward the channel exit at different speeds. However, most AF4 analysis tools have been optimized for other biological objects with limited sensitivity and throughput for exosomes separation.

In this paper, we implemented the process of separating the exosomes from the AF4 system on the simulation software. The results in our work verify that AF4 technique has great potential for exosomes separation. And provide guidance for the subsequent actual device fabrication.

## 2 Experiment

In this study, COMSOL Multiphysics® 5.2 software was used to separate proteins (bovine serum albumin, BSA) and exosomes. The technique used for the simulation is the previously mentioned asymmetrical flow field-flow fractionation (AF4). The separation is based on an ultrafiltration membrane that retains the solutes when a cross-flow is applied.

A 2D model was used to describe this system. Flow profile and samples migration are predominantly homogeneous along the breadth of the channel and that enabled to reduce the model to two dimensions. A mesh was created with very fine elements in the proximity to the bottom flat permeable surface (membrane) as samples migration



**Fig. 1** COMSOL 2D simulation model diagram. Since it is a long, flat channel, a partially enlarged picture is added at the top of the figure

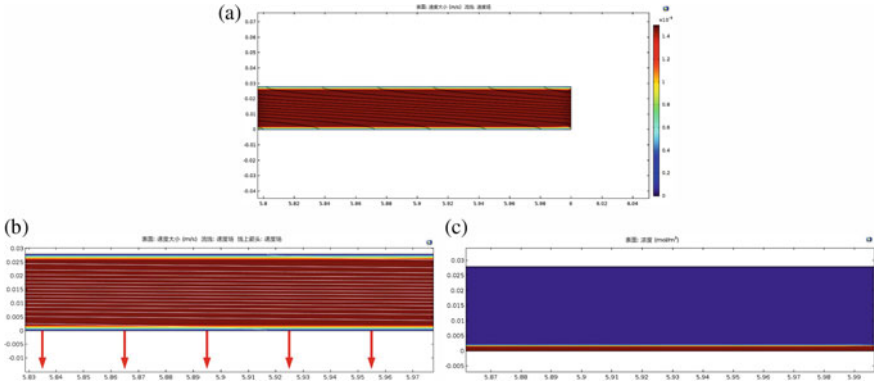
takes place in this region. A rectangle of 6 cm in length and 280  $\mu\text{m}$  in height was drawn as a separation channel (Fig. 1).

To describe the flow, single-phase laminar flow was used, and boundary conditions (inlet, outlets) were set to define channel flow and cross-flow. The study of the flow profile was solved as stationary-state problem, and this output was used to solve the time-dependent problem of samples migration. Transport of diluted species (convection and diffusion) was used to simulate samples of two different sizes, BSA and exosomes with hydrodynamic radius 3.7 nm and 20 nm respectively.

### 3 Results and Discussion

#### 3.1 Flow Rate and Concentration Distributions

After optimizing the parameters of the 2D flow rate stationary-state simulation, the obtained flow velocity and concentration simulation results and partial enlargement patterns are shown in Fig. 2. It can be seen from Fig. 2a that the flow velocity at the center of the channel is much larger than the flow velocity of the upper and lower walls of the channel, and the channel flow for carrying current is in the form of laminar flow (parabolic type). In Fig. 2b, at the lower wall of the channel, there is a vertically downward flow field, indicating that the cross-flow is present in the channel, and that the curve of the streamline can also be seen from the direction of the streamline in the channel. It is also the effect of cross-flow. In contrast, the velocity of the channel flow is much greater than the speed of the cross-flow, so that it does not change the general distribution of the fluid in the entire channel.



**Fig. 2** Two-dimensional stationary-state flow rate simulation and concentration results. The figure is the physics field distribution near the exit

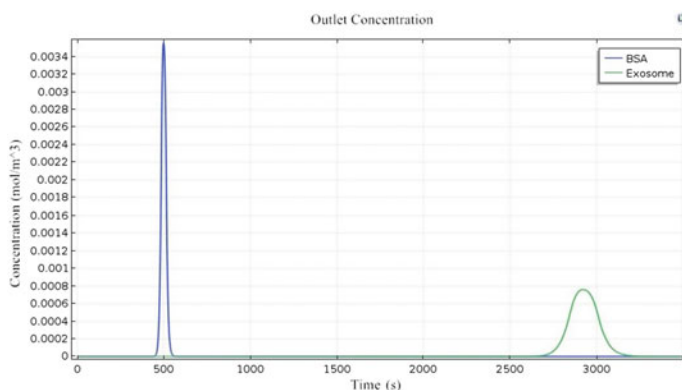
The distribution of sample concentration in the channel can also be represented by a two-dimensional stationary-state simulation (Fig. 2c). It can be seen from the simulation results that the sample is almost concentrated at the accumulation wall of the channel, again illustrating the effect of the cross-flow on the entire channel, and in the steady state, the concentration at the bottom of the channel entrance is almost zero. This is because when setting up sample injection, the concentration is injected into the channel as a pulse rather than a continuous injection channel.

### 3.2 Effect of Separation

The 2D distribution of flow rate and concentration can only observe some phenomena from the image, but it cannot quantitatively explain that the two samples with different diffusion coefficients have been separated. Therefore, a 1D function curve is needed to characterize the relationship of concentration over time at the exit of the channel.

In the simulation process, the transport of diluted species interface is used to enable 1D time-dependent analysis. Both samples enter the inlet at the same time with the Gaussian pulse. Since the different diffusion coefficients cause a difference in the transfer speed, at the exit, the two flow out of the channel at different times (Fig. 3).

According to the 1D function curve, it can be seen that the two substances exit the channel at different times at the exit. The blue line represents BSA, and the green one represents exosomes. Since the particle sizes of the two samples differ greatly, the time of the outflow channel is also quite different. This result is similar to the theoretical calculations, and the feasibility of the AF4 method for exosomes separation can be demonstrated using a simulation method.



**Fig. 3** 1D plot of export concentration versus time. The curves in the legend indicate two different colors representing BSA and exosomes

## 4 Conclusions

In this study, the theory of field-flow separation was applied to the separation of exosomes. Through theoretical analysis and simulation design, two-dimensional and one-dimensional simulation results were obtained, and the results were used to confirm that the method can separate exosomes. A simulation platform is built for constructing the field-flow fractionation device. If the added physical field and sample are modified, the method can also be used for simulation. Moreover, the fabrication of the actual microfluidic separation device can play a role of auxiliary reference. By modifying the size parameters of the device and the parameters of each physical field interface, a more suitable physical device can be obtained.

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