Numerical design of thermophoresis phenomenon for exosomes population separation

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Abstract

Separation and characterization techniques are growing rapidly in the biomedical world and thermodifussion, as well as thermophoresis, is becoming a challenging subject [1]. This work presents a numerical study of an exosomes separator microdevice, where different populations of vesicles are separated by means of thermal gradients. Different geometrical models, temperature gradients and entry fluxes have been analysed in order to determine the trajectories of exosomes along the microfluidic device and extract different populations.

Keywords: *Exosome, Thermodiffusion, Thermophoresis, Microdevice, Separation*

1. Introduction

Exosomes are small (40-250 nm) vesicles secreted to the extracellular medium by most of cell types. These vesicles are formed by a lipid bilayer containing in its interior lipids, proteins, messenger RNA (mRNA), micro RNA (miRNA), metabolites and specific proteins stemming from the segregator cell [2]. In multicellular organisms the intracellular communication is held by means of extracellular molecules. The liberated vesicles join the receptors of other cells and introduce relevant information about their state [3].

Exosomes are defined of high potential interest for the development of biological markers used to identify pathologies as cancer, metabolic or cardiovascular diseases [4]. The internal composition can variate depending on the physiological state and in different stages of pathologies exosomes can contain biomarkers that permit to define the localization, type and severity of it [5]. However, the present isolation and purification techniques involve several tedious steps, and hinder the progress of the investigations into clinical application [6, 7]. With the aim of obtaining a fast, easy and cheap device, a microdevice has been designed, where thermal gradients are used to direct, stratify and separate different exosomes populations based on their size.

Thermophoresis is the particle transport driven by thermal gradients [8]. This phenomenon is an additional particle transport mechanism on top of molecular diffusion and the total mass flux can be defined as [9]

$$\vec{J} = -\rho D\vec{\nabla}c - \rho c_0(1-c_0)D_T\vec{\nabla}T \qquad (1)$$

Where D is the molecular diffusion coefficient, D_T is the thermal diffusion coefficient, T is the temperature and c is the concentration. When a colloidal suspension is located in a thermal gradient, the particles move toward the hot or the cold side at a uniform trawl speed $v_T = -D_T\nabla T$ reaching a steady state concentration. When particles move toward the cold side the suspension is determined as thermophobic, whether the opposite situation is called thermophilic [10].

Based on the thermophoresis phenomena, it is seek to control and direct the displacement of exosomes extracting different sized populations.

2. Numerical simulation

ANSYS Fluent 16.0 software has been used for the numerical simulations. The exosomes motion trajectories in different conditions have been analysed and the most suitable case has been chosen for the final device.

2.1 Flow domain

The different designs have been used during the simulation. The initial device has been a bidimensional rectangular channel in order to analyse the direction of the vesicles. In a second design, an exit has been located in the vesicles precipitation zone in order to extract a population. Finally, a second exit has been located to

extract two different populations. For the first device different channel heights have been analysed; 50 µm, 100 µm, 200 µm, 400 µm, 800 µm and 1600 µm. For the second and third geometry the most adequate has been used. A cytometer has been located in the entry in order to limit the entry zone of the particles as it can be seen in Figure 1.

Figure 1: Geometrical domain used for simulations.

2.2 Numerical model

The cases have been solved using the Euler-Lagrange approach. The fluid phase is considered as continuum and the dispersed phase is considered as secondary, taking a small volume fraction.

The suspended particles have been tracked in the laminar flow by the Eulerian-Lagrangian Method, using the Discrete Phase Model (DPM) in a two-way interaction. And trajectories of each particle are computed in a Lagrangian frame.

The continuous phase and the discrete phase exchange heat, mass and momentum but the interaction between particles have been ignored since the particle volume fraction is low. As the displacement of the particles has been directed by thermal gradients, thermophoretic force has been activated. For the thermophoretic coefficient determination in the software ANSYS Fluent a unit change has to be done. Based on the equation from article "Novel thermophoretic particle separators: Numerical analysis and simulation" [11] the thermophoretic coefficient of each particle has been calculated by a User Defined Function:

$$D_{T\ fluent} = 6\pi \cdot \mu \cdot T \cdot D_{T\ \exp} \cdot \frac{P_{DIAM\,(p)}}{2} \qquad (2)$$

Where μ is the viscosity, T is the temperature, $D_{T\exp}$ is the experimental thermophoretic coefficient and $P_{DIAM\,(p)}$ is the particle diameter. Moreover, as the introduced particles are submicron size, the Saffman lift force has been activated.

Once all forces have been activated and their correspondent values are introduced, the injection properties are defined. A surface injection of particles from 40 nm to 250 nm at 298.16 K has been set from the central inlet of the cytometer. An entry flow of 20000 particles/sec has been defined and the same velocity as the fluid has been set.

As well as the injection properties, the material properties have been defined. First the properties of the carrier fluid have been set and then the properties of exosomes. On the one hand the porter fluid or the PBS and on the other hand the Exosomes. Finally, the boundary conditions have been defined (Table 1).

Table 1: Simulation boundary conditions.

Boundary conditions	
Top wall	302.16 K
Bottom wall	294.16 K
Lateral wall	Adiabatic
Cytometer	298.16 K
Inlet Velocity	Case dependant
Outlet	Outflow = 1

With the aim of analysing the population separation, different entry fluxes for the sample and the porter fluid have been analysed. The $f_q = \frac{Q_C}{Q_T}$ parameter has been defined, the relationship among the flow of the central cavity of the cytometer and the total flow. The defined entry flow relationship values have been 0.2, 0.1, 0.05 and 0.025 for total flows of 50, 100 and 200 ml/min per channel height.

3. Results

3.1 Exosomes displacement tendency

It has been seen that in all simulation exosomes are stratified depending on their size and they all move toward the cold wall. The first particles reaching the cold wall are the biggest ones whether the last are the smallest ones.

The particles trajectory has been determined for each case and the obtained separation degree is bigger for high Reynolds number and smallest entry flow. Moreover it has been seen that the smaller is f_q, better is the separation. In order to select the case to work with posterior simulations the parameter time has also been taken into account. The 400 µm height and 100 ml/min entry flow case has been selected, with a separation time around 100 min (Figure 2).

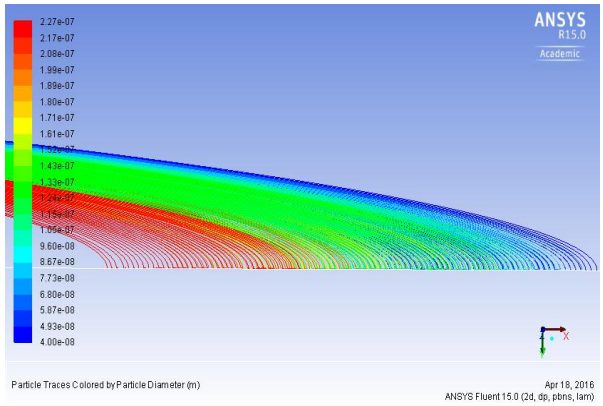

Figure 2: Particle displacement toward the cold wall.

3.2 Exosomes extraction

Once the suitable case has been selected, the extraction of the exosomes has been simulated. An outlet has been positioned where the biggest population of vesicles end with the aim of obtaining only the biggest vesicles. The 5% of the total flow has been directed to that outlet and the velocity has been adjusted to separate different populations. It has been seen that this technique gives variability to the user, giving the chance to adjust the velocity in order to separate the desired population.

If a single outlet is located in the inferior wall only two populations can be separated in each lap. In order to separate faster and obtain three populations in each lap, a second exit has been located next to the first one. This chance makes possible to separate three populations, but one of the flows of the outlets has to be taken to the limit and is not ideal for experimental procedures. It has also been possible to extract two different populations from the outlets of the inferior part of the device. This way, two concentred populations are obtained in each lap.

4. Discussion and conclusions

In this work, thermophoresis phenomenon has been numerically studied as an exosome population separator. First a bidimensional study of different microdevices has been done to analyse the vesicles behaviour in front of a thermal gradient. Once the movement pattern of exosomes has been defined, one exit has been positioned for particles extraction. Then, a second exit has been positioned in order to separate more populations in the same time. Regarding the extraction, it has been concluded that the single outlet device gives the option for variability and allows the user to adjust parameters to obtain the desired population. On the other hand, with a two outlets device, two more concentred populations can be obtained. With the aim of defining the most suitable device for the posterior manufacturing, different entry and outlet fluxes have been simulated.

All in all, it has been seen that thermodifussion is an easy, fast and cheap method for separation.

Taking into account all the conclusions, actually, we are working on the experimental validation of the device.

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