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Numerical simulation of cell squeezing through a micropore by the immersed boundary method

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Abstract

The deformability of cells has been used as a biomarker to detect circulating tumor cells (CTCs) from patient blood sample using microfluidic devices with microscale pores. Successful separations of CTCs from a blood sample requires careful design of the micropore size and applied pressure. This paper presented a parametric study of cell squeezing through micropores with different size and pressure. Different membrane compressibility modulus was used to characterize the deformability of varying cancer cells. Nucleus effect was also considered. It shows that the cell translocation time through the micropore increases with cell membrane compressibility modulus and nucleus stiffness. Particularly, it increases exponentially as the micropore diameter or pressure decreases. The simulation results such as the cell squeezing shape and translocation time agree well with experimental observations. The simulation results suggest that special care should be taken in applying Laplace-Young equation (LYE) to microfluidic design due to the nonuniform stress distribution and membrane bending resistance.

Keywords

Lattice Boltzmann Method; Cancer Cell Detection; Microfluidics Design; Immersed Boundary Method

1 Introduction

In USA, cancer has surpassed heart disease as the leading cause of death for young people (age less than 85)[1]. Metastasis, the spreading of cancer cells from the original tumor site to form a secondary tumor, accounts for about 90% of the death of cancer patients[2]. As cells transform into cancerous state, they become very soft because the organized cytoskeleton network is changed into an irregular one, thus, they can easily penetrate the surrounding tissue and blood vessels, and enter the circulation system (intravasation). So they are also named circulating tumor cells(CTC). Some of these cells can adhere to blood vessel walls and migrate into the local tissue(extravasation), where they can form a secondary tumor[3,

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4]. The reduced friction on the tumor cell surface may also contribute to the migration process[5]. Thus, cell deformability is an important biomarker to differentiate diseased cells from healthy cells[3, 4, 6, 7].

In many cases, a patient's chance of full recovery is much higher if the potential cancer is diagnosed and treated earlier. However, detecting CTCs is very challenging, because CTCs are rare, e.g., a few CTCs in millions of white blood cells and a billion of red blood cells (RBCs)[8] in 1 *mL* blood sample. Many different physical mechanism have been used to enrich CTCs, including size[9–12], magnetic field[13, 14], electric field[15, 16], optical force[17, 18], acoustic fields[19, 20], and microfluidic surface modifications[21–23]. Meanwhile, the specific binding between receptors expressed on cancer cell membrane and ligands coated on microfluidic chips have been explored to detect CTCs[24, 25]. Among these methods, they either require sophisticated cell preparation, careful microfluidics design, or external fields to enumerate CTCs. Alternatively, a low cost microfluidic chip based on cell deformability has been used to isolate CTCs[26, 27].

Based on the deformability differences, microfluidics with proper size of micropores or gaps have been used to differentiate cancer cells[11, 26–29] and even cancer cell clusters[30] from other cells. However, it is not clear what micropore size or proper pressure should be used to differentiate the cells efficiently. In this paper, we studied the cell translocation process through a narrow pore numerically, particularly with a focus on separating CTCs from white blood cells, as RBCs can be removed relatively easily based on their size and mass difference. The effect of cell deformability, the pressure difference, and the pore size on cell translocation time were studied using the combined lattice Boltzmann method and a coarse grained cell membrane model. The numerical results were also compared with experimental results reported in Ref[11]. It demonstrated the capabilities of the developed model to optimize the microfluidics design such that the cancer cells can be separated from other blood cells efficiently.

The remainder of this paper is structured as follows. The lattice Boltzmann fluid solver, the cancer cell model, and the fluid-structure interaction model is introduced in Section 2. Next, effect of membrane deformability, pressure and pore size on cell squeezing, and rational design of microfluidics are presented in Section 3. Finally, conclusions and future work are summarized in Section 4.

2 Methods

Simulation of cell squeezing through a micropore is nontrivial, as the cell undergoes large deformation under fluid shear. Freund studied the flow of red blood cells through a narrow spleen-like slit using a boundary integral model considering the effect of flow rate and cytosol viscosity[31]. Similarly, dissipative particle dynamics based model was also performed to investigate the splenic clearance of aged RBCs[32]. These two models are related to RBCs, not cancer cells. Zhang studied the passing of CTCs through microchannels with different 3D shapes using liquid droplet cell models[33]. However, this type fluid based cell model cannot model the bending of the cell membrane. Thus, in this study, a spring connected network model is used to model the cell where the stretching and bending

resistance were included. The fluid is solved by the Lattice Boltzmann method. The coupling is achieved through the immersed boundary method[34, 35]. This approach has been successfully applied to study blood flow[36, 37], drug delivery[38, 39], and verified in our previous publication[40].

2.1 Lattice Boltzmann fluid solver

As a competitive fluid solver, the lattice Boltzmann method (LBM) has been used extensively in fluid flow modeling [36, 41–44]. Interested readers on the underlying theory are referred to literature [45–48]. LBM was shown to be a second order accurate method in space and time [49]. The main concept of the LBM is the density distribution function $f_i(\mathbf{x}, t)$ in phase space $(\mathbf{x}, \vec{c}_i)$, where t denotes the time and $\vec{c}_i$ denotes the lattice velocity. The evolution of the density distribution function involves streaming and collision processes.

$$\underbrace{f_i(\mathbf{x} + \delta t \vec{c}_i, t + \delta t) - f_i(\mathbf{x}, t)}_{\text{streaming}} = - \underbrace{\frac{1}{\tau}(f_i(\mathbf{x}, t) - f_i^{eq}(\mathbf{x}, t))}_{\text{BGK collision}} + F_i \quad (1)$$

where the simplest Bhatnagar–Gross–Krook (BGK) scheme [50] is used for the collision term, F_i is the body force term[51] that will be used to represent immersed cell boundaries. The equilibrium distribution $f_i^{eq}(\mathbf{x}, t)$ is given by

$$f_i^{eq}(\mathbf{x}, t) = w_i \rho \left(1 + \frac{\vec{c}_i \cdot \vec{u}}{c_s^2} + \frac{(\vec{c}_i \cdot \vec{u})^2}{2c_s^4} - \frac{\vec{u}^2}{2c_s^2} \right) \quad (2)$$

where w_i is the weight coefficients, c_s is the speed of sound. The fluid density ρ and velocity $\vec{u}$ can be calculated as

$$\rho = \sum f_i, \quad \rho \vec{u} = \sum f_i \vec{c}_i + \frac{\delta t}{2} \vec{g} \quad (3)$$

Where $\vec{g}$ is the external force density vector, which is related to F_i as

$$F_i = \left(1 - \frac{1}{2\tau} \right) w_i \left(\frac{\vec{c}_i \cdot \vec{u}}{c_s^2} + \frac{\vec{c}_i \cdot \vec{u}}{c_s^4} \vec{c}_i \right) \cdot \vec{g} \quad (4)$$

The fluid viscosity ν can be related to the relaxation parameter τ as

$$\nu = c_s^2 (\tau - 0.5) = \frac{\tau - 0.5}{3} \quad (5)$$

2.2 Cancer cell model

To capture the mechanics of cancer cells, the cell membrane was modeled as particles interacting with potential energy. We treated the internal components as cytoplasm and assuming nucleus effect can be neglected. This assumption was also used by other reference[33]. The cell membrane is triangulated into a set of interacting particles X_i . Following previous blood cell and white blood cell models[52], the potential energy of the membrane contains stretching, bending, and the resistance to area and volume changes, as shown in Eqn.6. The stretching energy is used to represent the cytoskeleton's resistance to deformation. The bending represents the rigidity of the membrane bilayer.

$$V(X_i) = V_{stretch} + V_{bending} + V_{area} + V_{volume} \quad (6)$$

When cancer cells are squeezed through a micorchannel, the spherical cell shape is transited into a sausage shape(i.e., a cylinder with hemispherical caps at both ends). During this process, the volume of the cell is usually conserved but the effective cancer cell surface area is increased. A 52% area increase on average was observed during the cancer cell sphere to sausage transition[53]. The increased surface area is due to the unfolding of the microvilli on the cell surface. It is interesting that most of the cancer cells were dead after deformed from spheres with an average diameter of $16.5 \mu m$ to cylinders having a mean length of $53 \mu m$ in $7 \mu m$ capillaries[53]. The study of membrane rupture for cancers is deferred as future work.

The stretching potential consists of an attractive finitely extensible nonlinear elastic springs (FENE) and an repulsive power form potential (POW):

$$V_{stretch} = U_{FENE} + U_{POW} = \sum_{j \in 1 \dots N_s} \left[-\frac{k_s}{2} l_m^2 \log[1 - x_j^2] + \frac{k_p}{l_j} \right] \quad (7)$$

where l_m is the maximum bond length, the j th bond length ratio is $x_j = l_j/l_m$. The ratio $x_0 = 0.5$ is defined at the equilibrium. k_s and k_p are the related constants. Once k_s is given, k_p can be found using the value of x_0 where the net force is zero. Young's modulus can be derived through the Cauchy stress and strain relationship. The method to calculating the stress from particle based method is through the virial theorem[54]. The shear modulus and Young's modulus for cell network model are summarized in Ref.[55, 56]. For example, for the potential used in Eqn.7, the shear modulus μ is

$$\mu = \frac{\sqrt{3}}{4} \left(\frac{2k_s x_0^2}{(1 - x_0^2)^2} + \frac{3k_p}{l_0^3} \right) \quad (8)$$

The Poisson ratio ν of an isotropic triangular mesh is $1/3$ [55], thus, the Young's modulus E is

$$E = \frac{8}{3}\mu \quad (9)$$

The membrane thickness is about 100 Angstrom[57]. The thickness of the membrane should multiply Young's modulus to convert it into a 2D membrane elastic modulus. The corresponding Young's modulus for 2D membrane is shown in the third column in Table 1. It shows that the Young's modulus for cancer cells is very similar to Red blood cells, e.g., $E \in (15-36\mu N/m)$ [56, p.53].

Bending energy is defined

$$V_{bending} = \sum_{j=1 \dots N_s} k_b (1 - \cos(\theta_j - \theta_0)) \quad (10)$$

where k_b is the bending constant, θ_j is the instantaneous angle formed by the outward surface normals of two adjacent triangular meshes that share the same edge j . θ_0 is the corresponding equilibrium or spontaneous angle.

Area and volume based energy are defined as

$$V_{area} = \frac{k_g (A - A_0)^2}{2A_0} + \sum_{j=1 \dots N_t} \frac{k_l (A_j - A_{j0})^2}{2A_{j0}} \quad (11)$$

$$V_{volume} = \frac{k_v (V - V_0)^2}{2V_0} \quad (12)$$

where k_g, k_l are the global and local area potential constants; A, A_0 are the instantaneous and spontaneous total surface area of the cell membrane; A_j, A_{j0} are the instantaneous and spontaneous surface area for the j th triangle surface. k_v is the volume potential constant, V, V_0 are the instantaneous and the equilibrium cell volume.

The surface compressibility is used to characterize the deformability of the cells. The surface compression modulus K , defined as

$$K = - \left. \frac{\partial P}{\partial \log(A)} \right|_{A=A_0} \quad (13)$$

where P is the in-plane pressure given by $P = -\frac{1}{2}(\tau_{xx} + \tau_{yy})$, A is the surface area. Fedosov analyzed different spring connected network models and gave the relationship between K and other potential constants[56, p.43]. For example, for FENE and POW potential,

$$K = 2\mu_0 + k_g + k_l \quad (14)$$

where μ_0 is the shear modulus, and k_g, k_l are area conservation constants used in Eqn.11. Generally, $k_g + k_l \gg \mu_0$ is required for nearly incompressible spring network. In our model, different compression modulus were used to study the effect of deformability on the cell squeezing process.

Cancer cells have a lot in common compared to white blood cells. The cell bending stiffness for white blood cells is $500k_B T$, about $2 \times 10^{-18} J$ in temperature of $300 K$ [52]. The stretching modulus for white blood cells is $18.9 \mu N/m$. The deformability of cancer cells varies a lot. CTCs usually present a dormant state[58] where CTC is relatively stiffer. When they become malignant ones, the stiffness decreases significantly[5]. Many literature reported that the Young's modulus for cancer cells, as summarized in Table 1. Those data are measured using atomic force microscopy (AFM). Interested readers can find how to use AFM and its application to cancer cell research in Ref.[60]. The Young's modulus for other cell types has been summarized in Ref.[61].

2.3 The immersed boundary method

The immersed boundary method (IBM) was used to couple the fluid and solid motion because of the elimination of the expensive mesh regeneration step. Interested readers can refer to [34, 62] for details. Basically it ensures the velocity and surface force continuous conditions at the fluid-solid interface through interpolations. For immersed solid coordinates $\mathbf{X}$, its velocity $\mathbf{u}(\mathbf{X}, t)$ is interpolated from local fluid velocity $\mathbf{u}(\mathbf{x}, t)$ and the solid force $\mathbf{F}(\mathbf{X}, t)$ is spread out into the local fluid as force density $\mathbf{f}(\mathbf{x}, t)$,

$$\mathbf{u}(\mathbf{X}, t) = \int \mathbf{u}(\mathbf{x}, t) \delta(\mathbf{x} - \mathbf{X}) d\mathbf{x} \quad (15)$$

$$\mathbf{f}(\mathbf{x}, t) = \int \mathbf{F}(\mathbf{X}, t) \delta(\mathbf{x} - \mathbf{X}) d\mathbf{X} \quad (16)$$

where $\delta(\mathbf{x})$ is the delta function. In 3D numerical implementation, generally $\delta(\mathbf{x})$ is constructed as the product of one dimensional functions, e.g., $\delta(\mathbf{x}) = \phi(x)\phi(y)\phi(z)$ with $\phi(r)$ defined by as:

$$\phi(r) = \begin{cases} 0, & \text{otherwise} \\ \frac{1}{4}\left(1 + \cos\left(\frac{\pi x}{2}\right)\right), & |r| \leq 2 \end{cases} \quad (17)$$

The above $\phi(r)$ used 4 nearby nodes such that a solid node influences nearby 64 nodes in 3D. Other interpolations can be used as well, such as using 2 nodes or 3 nodes. Different choice of interpolation functions will impact the coupling accuracy, the influence range of the solid particles on the fluid, and the computational cost [63].

A 2D illustration of the velocity interpolation and force spreading process is shown in Fig.1 where the $\delta(x)$ only considers four nearby fluid nodes.

3 Numerical results

3.1 Validation: capsule squeezing

To validate our cell model, we simulated the capsule deformation when it was squeezed through a constriction slit and compared with experimental data reported in Ref.[64]. The size of the fluid channel was $L = 18 \text{ mm}$, $W = 7.5 \text{ mm}$, $D = 3 \text{ mm}$. The capsule was modeled as a spherical membrane with a diameter of $L_0 = 3.6 \text{ mm}$. There were two isosceles trapezoid shape blocks inserted at $L = 9.2 \text{ mm}$ along the channel, forming a rectangular gap with a width $w_c = 1.5 \text{ mm}$ and a depth $D = 3 \text{ mm}$. The short base and long base for each trapezoid was $l_a = 0.5 \text{ mm}$ and $l_b = 3 \text{ mm}$. The geometrical information was selected based on the experiments from Ref.[64]. The capsule membrane mechanics was described in Sec. 2.2 with a shear modulus of $9 \times 10^{-2} \mu\text{N/m}$. The bending stiffness was set to $2.3 \times 10^{-19} \text{ J}$, area coefficients $k_g = k_l = 21 \mu\text{N/m}$. In lattice Boltzmann units, the lattice space was $dx = 0.05 \text{ mm}$, relaxation parameter $\tau = 1$, and time step was $dt = 0.42 \text{ ms}$. The applied maximum velocity at the inlet was 0.01. The outlet was open. The Reynolds number defined using the maximum velocity and capsule diameter was $Re = 0.43$. The capsule was discretized into 10242 nodes, 30720 springs and 20480 triangle elements. The snapshot of the simulation of the capsule and the flow field can be seen at Fig.2a. The stream-wise capsule length L/L_0 was plotted as a function of membrane tip position $y_t^* = y_t/l_b$, as shown in Fig.2b. The position deformation curve agreed well with experimental data. It showed that the model can capture the transition of the capsule through the slit, as indicated by the sharp pulse of the curve, which validated the model.

3.2 Cancer cell squeezing model setup

In our model, the geometry of the microfluidic channel is $43 \mu\text{m} \times 30 \mu\text{m} \times 30 \mu\text{m}$, with a narrow pore of diameter of $10 \mu\text{m}$. The side view and left view of the channel is shown in Fig.3. The pore diameter is selected from $8 \sim 12 \mu\text{m}$ based on the suggestion from experimental work[11].

The pore size is also comparable to other narrow channel size used in microfluidics. e.g., a square cross section of $10 \mu\text{m} \times 10 \mu\text{m}$ was used in Ref.[27], or a cross section of $6 \mu\text{m} \times 15 \mu\text{m}$ in Ref.[5]. The critical channel diameter to filter CTC from RBCs in microfluidic channel is

reported between 5 and 12 μm [33]. Bounce back boundary conditions were applied at the walls. The inlet was applied a pressure of 15 Pa, while the outlet pressure was set at 0. The initial set up of the system is shown in Fig.4. The lattice size for the fluid is 0.5 μm . The cell has 2562 nodes and 5120 triangle surfaces with mean bond length of 0.56 μm . The diameter of the cell was selected as an average $L_0 = 15 \mu\text{m}$. The relaxation parameter $\tau = 0.9$.

The physical properties of the membrane are listed in Table.2. Notice that k_g is set to the same as k_f .

Cells with five different deformability properties(labeled as $K1, \dots, K5$ with compression modulus decreasing as the number increases, see Table 2) were studied in this work. The area coefficient was selected such that the Young's modulus was within the range of experimental data as shown in Table.1. Here $K = \frac{E}{3(1-2\nu)} = E$ where $\nu = 1/3$ was used assuming isotropic triangular mesh under small deformations[55]. The volume coefficient was selected to maintain a relatively constant cell volume, e.g., the cell volume change was about 0.1% during deformation. Except the case $K1$ where the cell was blocked by the pore, the cell translocation patterns were very similar. Here the $K3$ cell was selected for analysis. The time sequence of the simulation were shown in Fig.5.

As the cell approaches to the micropore, the strong viscous shear force would induce large cell membrane deformation. The cell membrane would form a protrusion at the membrane tip. Once the membrane tip passed through the micropore, it would expand at the other side of the pore. The tension distribution of the cell membrane is not uniform, with higher stress at the heading membrane and the membrane within the micropore. The tail of the membrane were shrunk first before it entered the pore, and then quickly stretched out and bend in the reverse direction once it reached at the other side of the pore. After the membrane tail left the micropore, the cell would keep expanding toward the wall until it reached a steady state, as shown in Fig.5d. The streamlines bended toward the wall sharply after the tailing membrane left the micropore. The changes of the streamlines can be seen from the comparison between Fig.5a and 5d.

3.3 Deformability effect on cell translocation

The snapshots of the simulation of cell with different area compression modulus squeezing through a narrow pore is shown in Fig.6. The gap formed between the cell membrane and the pore wall depends on the cell deformability, with larger gap for smaller membrane compression modulus. It is interesting to see that the cell with $K = 4020 \mu\text{N/m}$ could not pass through the pore, as indicated by the $K1$ (yellow line) in Fig.6c and 6d. The deformability also influence the curvature of the membrane for the steady parachute shape, with larger curvature for flexible membranes. Notice that the cell membrane penetrated into the wall for $K = 4020 \mu\text{N/m}$ when the cell was blocked. This is due to the large stiffness of the membrane. The focus of this paper is cell translocation through a micropore, not cell blocking. Thus, the model may not accurately simulate the case when cell membrane is in contact with channel wall. However, it does not change our main results on translocation time. The problem could be resolved if the fluid grid is refined or a repulsive force is applied

when the cell is approaching the wall, which requires a significant amount of work. Thus, modeling the cell contact problem under flow would be deferred to future work.

During the cell translocation process, the flow volume rate passed the cross section of the micropore can be used to characterize the translocation time. The flow volume rate is also related to the ionic current that can be directly measured through electronic nodes[11]. The conductivity of the micropore depends on the physical blockage by cells, thus it is proportional to the volume rate of the solution. The volume rate through the micropore ($x = 43$) was measured in all the simulations performed, as shown in Fig.7.

As shown from the figure, the volume flow rate decreases more than 80% as the cell passes the pore, resulting a sharp dip in the volume rate time history curve. The flow rate returns to the original level after the cell leaves the pore. The time of the blockage depends on the cell deformability, with shorter translocation time for softer cancer cells. This is consistent with the experimental data reported in Ref[11]. It is interesting to see that there is a second dip in the flow rate for less deformable cells. That is due to the tension increase in tail membrane of the cell. For example, for the cell case *K3*, the tension of the tail membrane at the peak volume rate (the data point at $t = 2.99ms$) is shown in Fig.8a, while the tension of the membrane at the dip in the flow rate(the point at $t = 3.67ms$) is shown in Fig.8b

To characterize the easiness of cells squeezing through the micropore, the translocation time of the cells were recorded. The translocation time is obtained from the volume rate time history at the middle section of the micropore(see Fig.7). The translocation time for different cell deformability is shown in Fig.9

The time for $K1 = 4020\mu N/m$ is not shown in the figure, because the cell is blocked at the entrance of the pore, the translocation time is infinity. For different cell stiffness, the translocation time is between $2000 \sim 5000\mu s$. The reported time for cell translocation for cancer cells is $138.16 \pm 192.4\mu s$. There is about an order of magnitude in difference. Notice that the micropore length used in [11] is 200 nm, which is 15 times smaller than $3\mu m$ used in the simulation. Consider these differences, we can conclude that the model can correctly reproduce the range of the cancer cell translocation time during experiments.

3.4 Pressure and pore size effect on cell translocation

Applied the pressure and the micropore size are the key design parameters for microfluidic size based CTC separation. Different pressure difference for cell with deformability $20\mu N/m$ (the case *K5*) squeezing through a $10\mu m$ micropore was studied. The pressure difference applied at the inlet is $5pa, 10pa, 15pa$, and $25pa$. The volume rate at the middle section of the micropore is shown in Fig.10a. To compare the volume change for different pressures, a normalized volume rate is plotted in Fig.10b.

It can be seen that the volume rate at different pressure almost all decreased by 80%. The pressure change does not influence the percentage of decreased volume rate. However, it does delay the translocation process as the pressure drops. The translocation time of the cells under different pressure is show in Fig.11.

As shown from the figure, the translocation time decreases with the increasing pressure. The translocation time decreases exponentially with pressure increase.

Similarly, the influence of micropore size on cell translocation time was also studied. The cell model used here has an area compression modulus of $20\mu N/m$. The applied pressure difference is $15pa$. Three difference micropore diameters are $8\mu m$, $10\mu m$, and $12\mu m$ in the study. The time history of the flow volume rate at the middle of the micropore is shown in Fig.12a. Since the flow volume rate is different for different pore size, the normalized flow rate with respect to its initial value is plotted in Fig.12b for comparison.

The volume change with larger pore size is more significant than smaller ones, as shown Fig.12a. However, the normalized volume change doesn't change a lot, as shown in Fig.12b, which is similar to the cases with different pressure differences. The translocation time for different micropore size is shown in Fig.13.

A further analysis reveals that the area formed by the volume change curve is equal to the cancer cell volume, see Fig.10a and Fig.12a. If the volume rate change is larger, the elapse time is shorter. From experimental point of view, e.g., the ionic current detection when cells passing the pore, see Ref.[11], larger volume rate change means it is easier to detect the cancer cell through the ionic current signal, but it requires a higher electrical signal sampling frequency, as the translocation time is shorter. Thus, this new analysis can be used to design such experiments.

3.5 Nucleus effect on cell translocation

Cancer cells usually showed enlarged nuclei, increased cellular deformability compared with normal cells[5, 65, 66]. The effect of the nucleus on cell squeezing through $10\mu m$ pore would be briefly discussed here. The cell membrane with area compressibility modulus $K_2 = 420\mu N/m$ was used. Another membrane was created inside the cell membrane to represent the nucleus, as shown in Fig.14a. The nucleus model used was exactly the same as the cell membrane model but with a diameter of $6\mu m$ and different shear moduli, e.g., $50\mu N/m$ and $500\mu N/m$, respectively. The applied pressure was $15 Pa$. The normalized volume rate for different is shown in Fig.14b. It showed that the cancer cell translocation time with a nucleus increased compared with the model without nucleus membrane. The translocation time also increases with the stiffness of the nucleus membrane, which is consistent with previous results as shown in Fig.7. Notice that the pore size is bigger than the nucleus size in the current model. When the pore size is smaller than the nucleus, an appropriate contact model should be considered, which will be deferred to future work.

3.6 Rational design of microfluidic devices for cell separation

The ultimate goal of the study is to provide rational design principles of microfluidics such that they could be used to efficiently to separate cancer cell from other cells in the blood sample. Two very important parameters in cell separation design are the pressure difference P and the diameter of micropore d . For example, what is the minimal pressure difference P required to squeeze the cells given the cell physical parameters and micropore size d ? What is the minimal pore size under given pressure difference? The following section is trying to give an example of how to apply Laplace-Young model (LYM) incorporating

numerical data (e.g., cell local curvature and stress) to design microfluidics, and discuss when it should be cautious to use LYM. Consider an equilibrium case when the micropore is blocked by the cell, the leading and tailing membrane could be treated as spherical caps, while the middle part of the membrane could be treated as a cylinder, as shown in Fig.15.

The cell bending stiffness is much smaller than the stretching stiffness, thus the membrane equilibrium conditions could be reasonably assumed to follow Laplace-Young equation. Using a modified Laplace-Young equation[28, 67], we have

$$\Delta P = 2\gamma\left(\frac{1}{r} - \frac{1}{R}\right) \quad (18)$$

where R and r is the radius of the bigger and smaller hemisphere, respectively. γ is the mean tension over the membrane. Noted here that the stress distribution over our cell model is not exactly uniform. For simplicity, the tension is assumed to be uniformly distributed over the whole membrane. From the conservation of volume, the volume of the cell under deformation (see Fig.15) should be equal to its initial volume, we have

$$\frac{\pi(R + \sqrt{R^2 - r^2})}{6}(3r^2 + (R + \sqrt{R^2 - r^2})^2) + \pi r^2 l + \frac{2}{3}\pi r^3 = V_0 \quad (19)$$

The surface area can be calculated as

$$2\pi R(R + \sqrt{R^2 - r^2}) + 2\pi r l + 2\pi r^2 = A \quad (20)$$

The ideal case is considered here that the smaller spherical cap is exactly a half sphere with radius $r = d/2$ where d is the micropore diameter. From Eqn. 19 and 20, with the assumption $r = d/2$, we can find the solution for R and l . For example, in the $K1$ case shown in Fig.6, we find that $R = 7\mu m$, $l = 2.3\mu m$, the minimal required pressure difference $P = 25pa$, which is about 67% higher than the pressure difference specified at the inlet $P_0 = 15pa$. The difference could be due to several reasons. First, the cell membrane model used here does resist bending, which is not considered in the Laplace-Young equation (18). Second, the assumption of a uniform tension distribution over the surface is not exactly correct. The tension distribution of the cell membrane is shown in Fig.16. It clearly shows a high stress region in the middle part of the membrane within the micropore. The heading and tailing membrane have relatively small tension. Thus, the uniform tension distribution assumption is not correct. To consider the nonuniform distribution of the tension, the local tension were used to predict the pressure difference. The local regions used to calculate the tension were defined as two hemispheres at the heading and tailing membrane. This approach is also consistent with the conditions that modified Eqn.18 is derived from.

With local surface averaged tension $\gamma = 128.2\mu N/m$, the predicted pressure $P = 14.4pa$, which is reasonably close to the applied pressure $15pa$. Thus, through the FSI code, we could calculate the minimal pressure required to squeeze through the micropore. Similarly,

the micropore size could also be optimized under given pressure difference and cell physical properties. However, if the bending resistance is relatively not small compared to tension, the LYM should not be used.

4 Conclusions

This paper studied the cancer cell squeezing through a micropore numerically by the immersed boundary method. The fluid motion was simulated by the lattice Boltzmann method, while the cell model was based on the spring connected network with stretching and bending resistance. The model was validated with a capsule squeezing through a rectangular slit. The deformation profile agreed well with the experimental work in [64]. Cells with different elasticity (membrane compressibility modulus) were squeezed through the micropore. We found that the cell translocation time increased with the cell membrane compressibility modulus. It also increased with nucleus stiffness. It increased exponentially as the micropore size or pressure decreased. Thus, the size of the micropore and the pressure difference become the two key parameters in microfluidic design. The Laplace-Young equation was widely used to analyze the cell curvature, the pressure and membrane tension. The study showed that the tension of the cell membrane was not uniform during the cell squeezing process, with high stress on the leading membrane and the membrane contacting the wall. This is different from the uniform tension distribution assumption in the Laplace-Young equation. However, when the membrane bending is relatively small, and a local averaged tension is used, the Laplace-Young equation can still provide a good prediction for the minimum required pressure based on the curvature. In the future, large scale simulation on cancer cell separation from blood sample will be performed to optimize the microfluidic chip design.

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Figure 1:

A 2D illustration for the velocity interpolation and force spreading process used in the immersed boundary method. Fluid nodes (black squares), solid nodes (red circles). (a) The solid velocity $\mathbf{u}(\mathbf{X}, t)$ is interpolated from four nearby fluid nodes enclosed by the dash line. (b) The restoring force $\mathbf{F}(\mathbf{X}, t)$ induced by the solid deformation is spread out to the four nearby fluid nodes enclosed by the dash line.

Figure 2:

(a) The snapshot for the capsule squeezing through a constricted slit. The channel size was $L = 18\text{ mm}$, $W = 7.5\text{ mm}$, $D = 3\text{ mm}$. Two isosceles trapezoids were placed at $L = 9.2\text{ mm}$ to form a rectangular slit. The capsule was a sphere with diameter of 3.6 mm . The background color showed the fluid velocity field. (b) The deformation of the capsule as it moved through the slit. The streamwise length of the capsule L/L_0 was plotted as a function of the membrane tip position $y_t^* = y_t/l_b$, where $y_t^* = 0$ indicated the center of the slit along streaming direction. The experimental data was obtained from Ref.[64, Fig.3]

Figure 3:
The geometry of the channel with narrow pore for cell squeezing test.

Figure 4:

The initial setup of the cancer cell squeezing through a narrow pore. The cell with a diameter of $15\ \mu\text{m}$ was positioned at $10\ \mu\text{m}$ away from the inlet. The pore size is $10\ \mu\text{m}$ in diameter and $3\ \mu\text{m}$ in length located at $x = 20\ \mu\text{m}$. A $15\ \text{Pa}$ pressure difference was applied at the inlet and right outlet. The background color shows the velocity distribution.

(a) $t = 0.33ms$ (b) $t = 1.67ms$ (c) $t = 2.67ms$ (d) $t = 4.33ms$ **Figure 5:**

Snapshots of cell squeezing through the micropore at different times. Velocity magnitude (background, in LB dimensionless units), streamlines (yellow), membrane tension are shown in the figure.

Figure 6:

Snapshots of cell squeezing through a narrow pore at different time. Cell profiles in the plane sliced through the cell center are shown in the figures. Cells with different deformability (area compressibility modulus K , in units of $\mu N/m$) are shown in different colors. Yellow: $K1 = 4020$, Blue: $K2 = 420$, Red: $K3 = 60$, Green: $K4 = 24$, Black: $K5 = 20$. The background color shows the velocity distribution for the $K2$ case. The fluid distribution for cells with different deformability shows similar pattern. They are not shown here.

Figure 7:

The flow volume rate passing through the micropore for cells with different deformabilities.

Figure 8:
Tension changes in the tail membrane after the cell exits the micropore.

Figure 9:
The translocation time for cells passing through a micropore with different cell membrane compression modulus.

Figure 10:

The volume rate at the middle section of the micropore under different pressure difference for cells with deformability of $20 \mu N/m$ squeezing through a micropore with diameter of $10 \mu m$. (a) volume rate; (b) normalized volume rate.

Figure 11:
The translocation time under different pressure for cells with deformability of $20\mu N/m$ squeezing through a micropore with diameter of $10\mu m$.

Figure 12:

The volume rate at the middle section of the micropore for different pore size for cells with deformability of $20\mu\text{N/m}$ squeezing through a micropore under pressure difference of 15pa . (a) volume rate; (b) normalized volume rate.

Figure 13:
The translocation time through different micropore size for cells with deformability of $20\mu N/m$ under the pressure of $15pa$.

(a)

(b)

Figure 14:

(a) A snapshot of cancer cell with a nucleus squeezing through a $10\mu\text{m}$ pore. The nucleus diameter is $6\mu\text{m}$, shown in red. (b) The normalized volume rate for the cancer cell with a nucleus at different nucleus membrane shear modulus.

Figure 15:

The micropore is blocked by the cells. The head and tail membrane form two curved surfaces (curves shown in the figure) that can be approximated as spherical caps with different radius r and R . The middle part of the membrane could be approximated as a cylinder with length l and radius r .

Figure 16: Nonuniform tension distribution over the cell membrane. Concentrated high tension was observed on the membrane within the pore. The tension in $\mu\text{N}/\text{m}$ are shown in the color bar.

Table 1:

Young's modulus for different type of cancer cells

Cell line	Young's modulus(kPa)	Young's modulus(2D, $\mu N/m$)	Reference
BC3726	1.4(± 0.7)	14(± 7)	[59]
T24	1.0(± 0.5)	10(± 5)	[59]
Hu456	0.4(± 0.3)	4(± 3)	[59]
HeyA8	0.71(± 0.53)	7.1(± 5.3)	[26]
MLCs	N/A	413(± 15.2)	[28]

Table 2:

Cell membrane properties for all the simulations performed in cells squeezing test

ID	$k_s(\mu N/m)$	$k_b(J)$	$k_g/k_f(\mu N/m)$	$K(\mu N/m)$	K_v
K5	5	2×10^{-19}	0	20	1×10^4
K4	5	2×10^{-19}	2	24	1×10^4
K3	5	2×10^{-19}	20	60	1×10^4
K2	5	2×10^{-19}	200	420	1×10^4
K1	5	2×10^{-19}	2000	4020	1×10^4