Simulation of the Transport of Biological Cells in the Microfluidic Device

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ABSTRACT

We developed a computer-aided methodology – the Bio-Particle (BP) simulation technique – for the general particle movement in Lab-On-A-Chip devices. This has also been validated with experiments on biological cells (3T3 cell; diameter 10 μm). The cell motion under steady flow was calculated by applying the one-way coupled Lagrangian method. The equation of motion consists of multiple force terms, i.e. drag, pressure gradient, Brownian random, and gravitational forces. By solving the governing equation with the Rosenbrock method based on an adaptive time-stepping technique, the cell trajectory can be solved over a prescribed 3D microfluidic device model. Moreover, each cell was assumed to be a solid sphere with adjustable elasticity, while the physical interactions between cells and device structures were also considered, particularly when cells sediment in the cell trapping sieves. Cells were trapped in these apertures where hydrodynamic forces were strong enough to resist cell movements. The result of experiment showed good agreement with that of the corresponding simulation. Finally, we have achieved an optimized structure of cell trapping chamber which showed the most enhanced cell trapping capability by using the Bio-Particle (BP) simulation technique.

Keywords: cell, trap, simulation, trajectory, microfluidics

1 INTRODUCTION

Researches on cellular analyses have been great interests in the "lab-on-a-chip" (LOC) technology over the past two decades. Recently, highthroughput analysis for the manipulation of large number of cell in the microfluidic device has been implemented to the biochemical analysis such as toxic screens on living cells [1].

Generally, to perform a variety of biological assays, several processes of cell analysis, such as sampling, trapping or sorting, treatment etc, are required. Among processes of cell analysis, cell trapping includes transport and controlling of minimum quantity of cell and reagents as a fundamental one. Requirement of uniform distribution of cells, i.e. uniform cell trapping, in the microfluidic systems is important in view of consistent results after chemical analysis.

Several methods have been introduced to trap cells under the steady state of flow condition. For example, dielectrophoresis (DEP) and optical tweezers (OT) etc, and these methods must be balanced with the Stokes drag, pressure difference due to fluid motion. DEP force refers to the force on induced polarization of particles in a nonuniform electric field [2], and OT refers to the force based on radiation pressure exerted by a strongly focused light beam. [3]. However, experiments adopting these external forces strongly depend on sophisticated controls of biological cells which are complicated by physical property of cell and medium. [4] Nevertheless, these methods have a uniqueness to trap specific cells.

In this study, we introduce a novel design for trapping cells using U-type sieves in the micro chamber. Utilizing the method illustrated in this paper, there is no need for employing any equipment to exert external force. Furthermore, we developed a computer-aided methodology – the Bio-Particle (BP) simulation technique –to visualize the cell movement in the LOC and the real-time processing. Using this approach, optimal design of the cell trapping chamber can be found.

2 METHOD

2.1 Geometrical Modeling

Computational model in the microfluidic system was generated by using several procedures. Firstly, 2-D pattern of microchannel was drawn by using an AutoCAD software. Secondly, 3-D STL (stereolithography) file format was made by the extrusion of 2-D drawing. Finally, STL file was imported through an automeshing software (PRO-AM, CD-adpaco) to generate refined meshes shown in Fig. 1A. Except inlet and outlet boundaries, all the wall boundaries were shrunk to make extrusion layers from the subsurface.

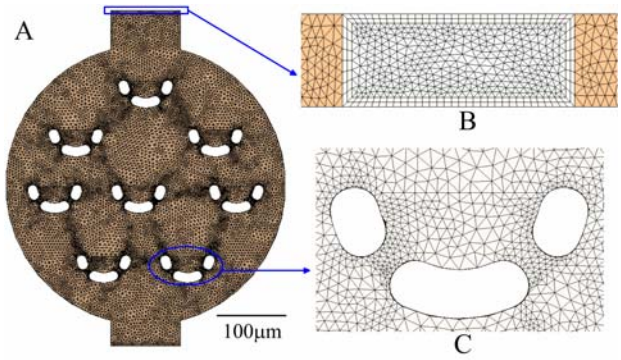

Figure 1: 3D Computational mesh used in the simulation; (A) overall number of grids is 759,000, (B) two types of grids (tetrahedral and prism shapes) were implemented, and (C) refined grid structure was used in apertures of cell trapping sieves.

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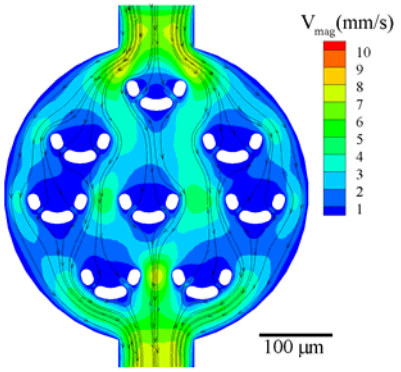

Figure 2: Theoretical flow velocity profile through single chamber with eight cell sieves. Velocity in cell sieve is significantly reduced compared to outside sieve, and eight low flow velocity regions are formed within the chamber [8].

Fig. 1B explicitly shows these interfaces composed of two types of meshes (tetrahedron and prism shapes) at the inlet boundary. To consider interaction between cells and the wall, especially apertures in sieve, very refined meshes were preferred as a minimum value of 1 μm size (Fig. 1C). The dimensions at the inlet was 100 µm wide × 40 µm high (W×H), and diameter of chamber was 400 µm. Each chamber has 8 sieves, and each sieve has two apertures (8 µm). The size of aperture of the sieve was selected less than averaged cell size (10 µm) (Fig. 1C).

2.2 Fluid Modeling

Computational fluid dynamics (CFD) simulations were carried out using commercial CFD tool (STAR-CD version 3.15a, CD-adpaco), which is based on finite volume method (FVM). As for working fluid, aqueous solution was selected to transfer cells into patterned micro-chambers. Momentum equations and continuity equation were solved using SIMPLE (Semi-Implicit Method for Pressure Linked Equation) algorithm with tolerance of 10^{-5}. As for numerical scheme, UD (upwind Scheme; 1st order of accuracy) for the spatial discretization were used with residual tolerances of each velocity component and pressure of 0.1 and 0.05, respectively. Aqueous solution with cells was supplied with a flat velocity profile at a uniform flow rate of $1\mu l/\min$. At the wall, no slip boundary condition was imposed.

2.3 Cell Modeling

Transient motion of every cell was calculated by using the *Lagrangian* approach for the pre-computed Newtonian flow fields. This includes spatial interpolation of the flow velocity at the cell's position. A cell was assumed to be a solid sphere, and interactions between cells were not

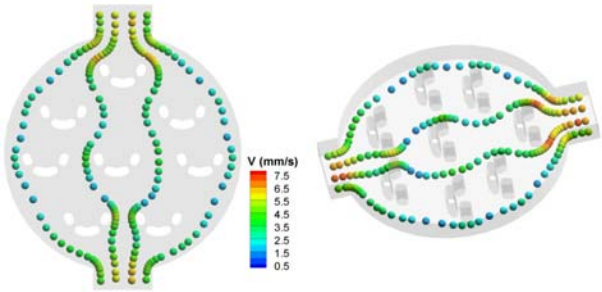

Figure 3: Selected Biological cell trajectories in 3D microfluidic chamber.

considered because solution with 3T3 cells was sufficiently low concentration ($1000/mm^3$). The forces considered in the equation of motion for the cells were the Stokes drag, the pressure gradient, diffusive, spring elastic and gravitational forces. Cell trajectories code was validated with the experiment results of the annular expansion channel [5, 6], and inclusion of external forces such as Stokes drag and the pressure gradient terms for the simulation had shown in good agreement with the experiment. Then, the resulting cell motion equations for seeking particle velocity ($\vec{u}_p$) and location vector ($\vec{x}_p$) are expressed as:

$$\frac{d\vec{u}_p}{dt} = \frac{\left(\vec{u}_f - \vec{u}_p\right)}{\tau_p} + \alpha\frac{D\vec{u}_f}{Dt} + \frac{\vec{F}_R}{m_p} + \frac{\vec{F}_S}{m_p} + (1-\alpha)\vec{g} \quad (1)$$

$$\frac{d\vec{x}_p}{dt} = \vec{u}_p \quad (2)$$

where $\tau_p\left(=\rho_p d_p^2/18\eta\right)$ is the particle response time to changes in the flow field, and $\alpha\left(=\rho/\rho_p\right)$ is the density ratio of the fluid to the particle. Here, ρ_p is the particle density of $1.040\,\mathrm{g\,cm^{-3}}$, and d_p is the particle diameter of $10\,\mu\mathrm{m}$. Every particle trajectory is obtained by integrating Eqs (1) and (2) under given initial conditions. Initial cell velocity was interpolated using fluid velocity at initial cell's seeding location. $\vec{F}_R$ indicates Brownian random force which is induced by collisions between microparticles and fluid molecules. Brownian random force was based on the Monte Carlo method which mimicking the random walk of particles. $\vec{F}_S$ is spring force term which induced by elastic collisions between spherical cells. A fourth order Rosenbrock method based on an adaptive time-stepping technique was utilized as the integration method because it is more reliable for stringent parameters than the Runge–Kutta method [7]. This adaptive time-stepping technique was automatically applied at locally high velocity regions, i.e., inlet, outlet and outer sieves, where shorter time steps were required to capture cell movements in detail (Fig. 3).

3 RESULTS AND DISCUSSION

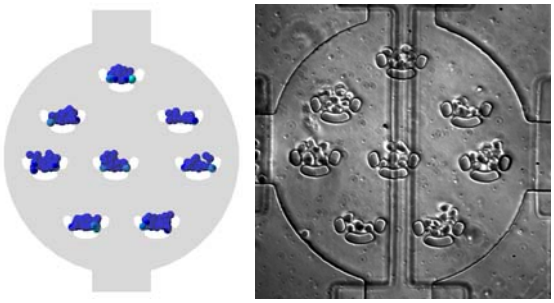

Figure 4: Theoretical (A) vs. experimental (B) seeding of HeLa cells in U-shaped sieve arrays within each microchamber [8].

The cell trapping microfluidic devices which were utilized here had been designed to satisfy two given conditions, namely, rapid isolation of cells from the bulk solution and uniform distribution of the trapped cells in each of the microchambers. Isolating cells from the bulk solution was undertaken by employing hydrodynamic suction at the aperture of the sieve. Cells were allowed to accumulate in the given microchamber until the capacity was reached. Following that, flow fields around the sieve would get diverted, which, in turn, would cause the incoming cells to move to another sieve in the chamber. This way, uniform distribution of cell trapping was achieved.

To optimize the design of cell trapping chamber, the streamline overlaps were taken into account. Here, the first sieve was designed to avoid the overlap of streamlines (Fig. 2). As a result, streamlines were diverted around the corresponding sieve, and cell trappings in a center-located sieve were successful. There was a dramatic increase in the number of trapped cells during the loading time of 60 sec. The cells were loaded in an essentially uniform manner, where there were 11.2±1.4 HeLacells/sieve and 11.5 ±2.4 BALB/3T2 cells/sieve; for the theoretical simulation, 15.2± 0.72 cells/sieve were employed [8]. Figure 4 shows both the theoretical and the experimental distribution of HeLa cells in a single microchamber. In the experimental part, the number of trapped cells was lower than expected which could be attributed to several factors not included in the model, including individual cell size variation and small cell clumps (2-3 cells) in the cell suspension loaded into the chip.

A series of dynamic images of the cell loading simulation were shown in Fig. 5. At cell loading time of 5 sec (Fig. 5A), a few cells were delivered into the chamber from the inlet. In Fig. 5B, further cells were trapped and piled in every sieve in the chamber; an average cell per a sieve was 12. Fig. 5C shows saturated state of cell loading process. This noticeably gives the uniformity of trapping cells in all the sieves. This simulation suggests that suitable cell loading time for corresponding experiment is estimated about 60 sec under the specific flow rate of $1 \mu l / min$ and low cell concentration ($1000 / mm^3$).

4 CONCLUSION

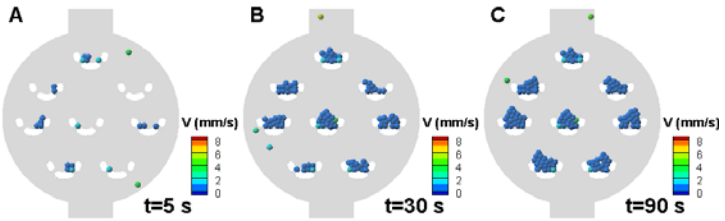

Figure 5: Cell loading simulation in one chamber. A few cells are trapped in surrounding sieves at cell loading time 5 sec (A); further cells are piled in the corresponding sieves at cell loading time 30 sec (B); all the sieves shows the uniformity of trapping cells (C) [8].

We have developed bio-particle (BP) trajectory code using computer-aided design method as mentioned in Methods section. This code has been considered to calculate motion of cells in CFD grid types such as tetrahedron and triangle prism. Thus, it can be regarded as a general code for the computation of biological cell movements in the practical geometry of the LOC. Moreover, it will be economical design tool before fabricating devices or running cell experiments; that is, trial and error in the computation will save the cost and time in the experiment.

Using the BP trajectory code, we designed micro-fabricated chambers for trapping cells. Through the movie clips of cell movements, we achieved optimized structure of cell trapping chamber which showed most enhanced cell trapping probability.

5 ACKNOWLEDGMENTS

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