Design of Fluidic Obstacles for Maximized Shear Forces to Induce Cell Lysis

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Abstract

Fluidic obstacles to lyse cells without clogging are modeled and designed in a SOI process. Obstacles are arranged to induce shear stresses in cells passing through the channels. A chemical treatment of the channel walls is also performed to prevent cell and biomolecule adhesion to the surfaces. Digital Particle Image Velocimetry (DPIV) and cell lysis measurements are proposed to quantify obstacle performance.

Introduction

Although the human genome has been sequenced the function of each encoded protein is not known. Many steps are required to isolate large amount of proteins in order to understand protein function. Functional genomic studies require cell sorting, lysis and biomolecule collection. Also, many diagnostic assays require lysis of cells in order to access some intracellular signal.

Several methods have been used to lyse cells in microdevices including chemical reagents and electroporation [1-3]. Sonication as a mechanical method for cell lysis is often used on the macroscale, however, mechanical lysis has not been pursued within microdevices. Mechanical lysis has advantages over other methods in that no detergents are necessary, which interfere with some assays and separations. Also, no external electrodes are needed, leading to an easier fabrication process. However, just flowing cells through a grating with sieve distance less than the cell diameter may not necessarily lyse cells [4].

Obstacles arranged in microfluidic channels in offset positions have the potential to create higher shear stresses since high velocity gradients are present. Also, the width of openings between obstacles can be larger than the cell diameters, which could lead to less clogging after lysis. A simple SOI single mask process with a bonded glass cover is used to create enclosed channels with obstacles to test shear lysis. A model is developed to calculate velocity fields and maximum shear forces for different obstacle geometries.

Theory

A model for optimizing shear-induced lysis using obstacles within a microfluidic environment is developed. Cells traveling through the obstacle field (see Fig. 1) will be subject to shear stresses that the bulk fluid will undergo to maintain flow. The larger the shear stresses the higher percentage of cells lysed as the plasma membranes are torn from underlying cytoskeleton.

Several assumptions must be made in order to apply the Navier-Stokes equations to modeling the velocity fields and thus shear forces experienced by cells passing through the micro-obstacles. The cell solution is assumed to be Newtonian, which is valid if the cell concentration is maintained at a low level. The fluid is also considered incompressible and the flow is laminar and steady. Maximum velocity gradients are assumed to be at the boundaries of the contractions and to simplify calculations only velocity profiles in these areas are derived.

The coordinate system used is right-handed rectilinear as seen in Fig.2 with \underline{e}_3 into the plane. Starting with Eq. 1 (Navier-Stokes in indicial notation) and with negligible body forces and steady incompressible flow Eq. 2 is reached.

$$-p_{i}+(\boldsymbol{l}+\boldsymbol{m})v_{k,ki}+\boldsymbol{m}_{i,kk}+\boldsymbol{r}b_{i}=\boldsymbol{r}\dot{v}_{i}(1)$$
$$-p_{i}+\boldsymbol{m}_{i,kk}=0$$
(2)

Only the narrowest direction in the obstacle channel (x_1) is considered since this is where the highest velocity gradient is present. Using Eq. 3 for high

aspect ratio rectangular channels along with conservation of mass and the previous simplification, Eq. 4 is arrived at. No slip boundary conditions were used such that v_2 is 0 at x_1 equals 0 and g. Also, the pressure drop was assumed constant through the gap.

$$Q_{local} = \frac{hg^3 \Delta P_{local}}{12 \, \text{m}} \tag{3}$$

$$\overline{v} = \frac{3Q}{hg^3} (x_1^2 - gx_1)\overline{e}_2$$
For $0 < x_1 < g$. (4)

Using Eq. 5 and the fact that the velocity gradient is highest at the boundaries, x_1 equals 0 or g, maximum shear stress in the \underline{e}_2 direction is solved for as a function of a flow rate in Eq. 6.

$$\boldsymbol{t}_{12} = \boldsymbol{m} \frac{dv_2}{dx_1}$$
(5)
$$\boldsymbol{t}_{\max} = \frac{3Q\boldsymbol{m}}{hg^2}$$
(6)

Finally, to solve the shear stress in terms of the pressure drop (Eq. 7) across the obstacles, volumetric flow rate was derived in terms of fluidic resistance per obstacle unit and number of units in series (Eq. 8)[5]. Where P is the pressure drop across the obstacles, g is the gap length between obstacles, l is the length of an obstacle, W is the expansion width after a contraction, h is the channel height, and N is the number of obstacle units consisting of 2 expansions and a 2 and 3 port gap. See Fig. 1 for some parameter descriptions. Q is the volumetric flow rate through the device and l is the viscosity of the fluid.

$$\boldsymbol{t}_{\max} = \left(\frac{10}{g^3} + \frac{24}{Wh^2}\right)^{-1} \frac{3\Delta P}{Ng^2 l}$$
(7)

$$Q = \Delta P \Big/ \frac{N m}{h} \left(\frac{10}{g^3} + \frac{24}{W h^2} \right) \tag{8}$$

Design

A SOI single mask process is used to define the channels and obstacles in a silicon wafer. A PyrexTM cover with reservoir holes is then anodically bonded to the wafer to enclose the channels. The PyrexTM wafer was drilled to form reservoir holes. In order to avoid surface adhesion of the cells to the surfaces of the channels or the PyrexTM cover, the surfaces are treated first with a silanizing reagant with a chemical structure ending in a methacryl group for an hour, washed with water, treated with polyacrylamide, and then washed again

with water [6]. This process polymerizes a neutral, inert monolayer on the surfaces, thereby sterically hindering adsorption of biomolecules.

For simplicity, we designed rectangular test structures with different offsets (Fig. 1). The gap g was varied from 12 to 30 micrometers, the length l from 9 to 30 micrometers, and the expansion width W from 60 to 140 micrometers. The entry channel diameter D was held constant at 100 micrometers. The test structures had scaled geometries so that there is also dependence between g, l, and W.

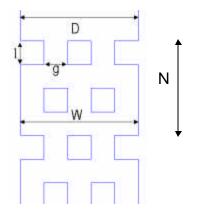


Fig. 1: Obstacle path for cell lysis. Test structures were designed with varied gaps (g), obstacle lengths (l) and expansion widths (W), D was the same at 100 microns for all test structures.

In addition to the derived theoretical model for maximizing shear stress, other considerations were noted when designing the device. Cells with an average diameter of 10 microns will clog the device if the gap size is much below 10 microns. This provides a lower limit on the gap size when optimizing the max shear stress. Also lithographic limits would provide a lower limit on the length of the obstacles.

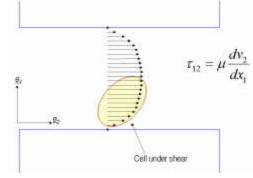


Fig. 2: Mechanism of cell shear lysis in cross sectional view. A non-negligible shear stress is induced in the passing cell by the high velocity gradient near the channel boundary at a contraction.

Experimental Design

Two experiments can be performed to test the physical properties of the rectangular test structures, one employing digital particle image velocimetry or DPIV to visualize the fluid flow profiles through the test structures and the other directly measuring the concentration of lysed cells.

DPIV has become an established technique for measuring fluid velocity profiles in microfluidic devices. The DPIV technique gives instantaneous, spatial measurement of the velocity observed in a planar cross section of flow by analyzing successive images of illuminated, seeded fluid flow using a video charge-coupled device camera [7,8]. Successive image pairs are cross-correlated to determine the velocity fields. Experiments comparing theoretical laminar flow profiles to DPIV imaged profiles of Newtonian fluid flow through contractions in channels have validated its use as an imaging technique [9]. Thus, DPIV will be suitable method for capturing velocity profiles for the obstacle test structures.

For the purposes of this study, DPIV can be used to image the velocity fields within the obstacle field. It will be important to observe the flow behavior at the surface of the obstacles, especially the corners in contact with head on fluid flow, because these are the regions with the highest anticipated shear rates and highest velocity gradients.

Three regions along the length of the device will be examined: the entrance to the obstacle region (N=0), the middle(N=2) and the exit (N=6) (Fig. 3). It will also be important to observe the flow behavior near the top (h=45um) and bottom (h=5um) of the channel in addition to the centerline (h=25um) because higher shear rates and higher velocity gradients are expected in these regions. Thus for each of the 12 test structures, three regions along the length at three different channel heights (9 total images) will be captured.

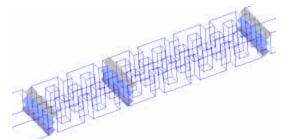


Fig 3: Cross sections of the obstacle channel to visualize with DPIV.

Measuring the concentration of lysed cells for the different rectangular layouts will give a comparative analysis of the different geometries. This can be done through a simple procedure using a Trypan blue exclusion assay. First, cell solution is collected after passing through the device. This solution is incubated 1:1 with Trypan blue for 5 minutes at 20 °C and then placed into a hemocytometer. Cells are counted in the grid areas of the hemocytometer, which specify a certain volume. Dead or lysed cells will be stained blue while live cells will exclude the Trypan blue. The lysis efficiency may be obtained from this cell count. The efficiencies for the 12 test structure geometries will be compared.

Analysis

Although no experiments will be performed for the purposes of this paper, the theoretical models can be used to examine the velocity flow behavior for the 12 test structure geometries. The theoretical maximum shear stress for each of the test structure geometries was calculated using Eq. 7 for different pressure drops across the channel (see Fig 4-6). While the channel height of 50um, number of obstacle units 6, and entry channel diameter of 100um were held constant for the calculations, the gap, the obstacle length, and expansion width were varied.

Based on the theoretical model, the shear stresses will be greatest for geometries with smaller gaps compared to obstacle lengths. In addition, for each of the three cases, equal gap and obstacle lengths, greater gap than obstacle length, and smaller gap than obstacle length, the geometries with the smallest features produced the highest shear stresses.

It is expected that the test structures with the highest theoretical shear stresses will have the highest experimentally measured concentration of lysed cells. Regardless whether cells are lysed using these test structures, imaging using DPIV will characterize the Newtonian fluid profile behavior through the obstacle geometry.

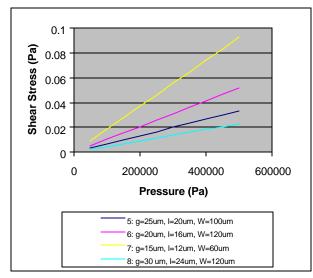


Fig. 4: Theoretical shear rates for test structures with equal gap and obstacle lengths.

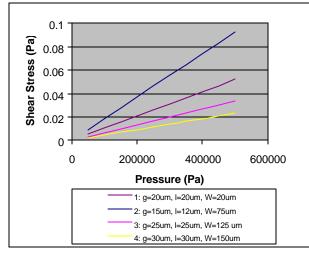


Fig. 5: Theoretical shear rates for test structures with greater gap compared to obstacle lengths.

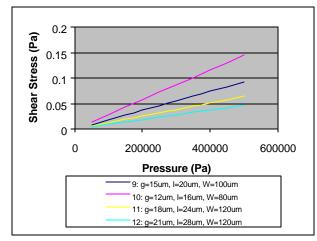


Fig. 6: Theoretical shear rates for test structures with smaller gap compared to obstacle lengths.

Future Work

Once the experimental data is obtained and correlations are established between theoretical and experimental observations, future work can be devoted to obtaining an optimal geometry. It is important to characterize the performance for the parameters held constant in this study, in particular the number of obstacle units and obstacle geometries. The latter will require new theoretical models for velocity and shear profiles. Further efforts can be focused on different obstacle geometries such as circular or triangular structures.

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