# Design of Microgroove in Micro-Flow-Channel for Cell Sorting

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**Abstract**

**Microgrooves on the bottom-surface of the micro-flow-channel have been designed to sort biological cells *in vitro*. The micro groove of the rectangular shape (0.0045 mm depth, and 0.2 mm length) has been fabricated on the surface of the polydimethylsiloxane (PDMS) disk by the photolithography technique. Variation has been made on the width (0.03 mm < w < 0.05 mm) of the groove. A rectangular flow channel (0.05 mm height × 2 mm width × 15 mm length) has been constructed between two transparent PDMS disks. C2C12 (mouse myoblast cell line) was used in the test. A flow velocity of the suspension of cells was controlled by the pressure difference between the inlet and the outlet. The shift movement of a cell along the oblique groove depends on the several parameters: the diameter of the cell, the width of the groove, and the velocity of the cell. The designed microgrooves in micro-flow-channel has availability for cell sorting.**

## Introduction

A flowing cell is captured to a wall of a flow path. Several cells adhere to the internal wall of the blood vessel, when the part of the wall has a defect *in vivo*. The morphology of the defect might govern the capture of cells. The capture also might depend on the property of the cell. Cancer cells adhere to the inner wall of the vessels. After transition from the original place to another place, the cancer cell proliferates to make the tumor tissue at the new place *in vivo*. The transition occurs through the blood vessels, or through the lymph vessels.

The effect of the surface property of the scaffold on the cell has been studied in the previous studies. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [1], and to simulate the morphology of the microcirculation [2]. The photolithography technique enables manufacturing the micro-morphology. The micro-fabrication technique has also been applied to design microfluidic systems *in vitro* [3]. Cells roll over on the surface of the wall in the shear flow, and make adhesion to the wall [4]. The surface was modified to capture flowing cells [5]. The technique was applied to handle cells in diagnostics *in vitro* [6]. The technique might also be applied to the cell sorter [7]. In the present study, the microgroove has been designed and manufactured by the photolithography technique to sort biological cells, and the movement of the single cell flowing at the microgroove has been analyzed *in vitro*.

## Methods

### Micro Grooves

For trapping cells, several micro grooves of the rectangular shape (0.0045 mm depth, and 0.2 mm length) have been fabricated on the surface of the polydimethylsiloxane (PDMS) plate with the photolithography technique. Several grooves are arranged on the same wall. At the groove arranged from upstream to downstream, variation has been made on the width of the groove: 0.03 mm, 0.04 mm, and 0.05 mm. The plate with micro grooves is the part of the flow channel (15 mm length × 2 mm width × 0.05 mm height), which is placed on the stage of the inverted phase-contrast microscope

### Flow Test

C2C12 (passage < 10, mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. Cells were cultured with the D-MEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS and 1% of Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin B, Life Technologies) in the incubator for one week before the flow test. The inner surface of the flow channel was hydrophilized by the oxygen (30 $cm^3$/min, 0.1 Pa) plasma ashing for one minute at 100 W by the reactive ion etching system (FA-1), and prefilled with the bovine serum albumin solution for thirty minutes at 310 K. Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM (Dulbecco's Modified Eagle's Medium). The suspension of cells (20000 cells/$cm^3$, 0.06 $cm^3$) was poured at the inlet of the flow channel. The flow occurs by the water head difference between the inlet and the outlet. The inlet hole (the height of 3 mm and the diameter of 5 mm) makes the pressure head.

Each cell rolling over the micro grooves on the bottom of the flow channel was observed by the microscope, and recorded by the camera, which is set at the eyepiece of the microscope. The movement of each cell was analyzed by "Kinovea" at the video images: 30 frames per second. At each image, the contour of each cell was traced with "Image J", and approximated to the circle to calculate the equivalent diameter. The velocity of each

cell around the groove was traced on the component parallel to the direction of the main flow of the velocity of the cell: immediately before the groove and in the groove. The travel distance of each cell along the groove was traced on the component (shifted distance) perpendicular to the main flow direction.

**Results**

The experimental results are as follows. Every cell travels along the groove to the downstream. The velocity of cells decreases with the time, because the pressure head at the inlet decreases with time. The velocity of each cell in the groove is slower than that before the groove. The velocity of each cell in the groove tends to be slower in the wider groove (0.05 mm). The shifted distance tends to increase with decrease of the diameter of the cell. The tendency is remarkable at the groove of the narrower width (0.03 mm). The shifted distance tends to increase with decrease of the velocity immediately before the groove.

**Discussion**

The rectangular grooves have been successfully manufactured on the wall of the micro fluid channel. The dimension of the grooves was confirmed by the laser microscope [8]. The micromachining technique has been applied to cellular technology. The microfluidic system was applied to sort biological cells, and to trap biological cells. The experimental results might contribute to analyze adhesive mechanism of cancer cell during metastasis. The micro trap might simulate adhesive mechanism of flowing cells.

Several fluid flow systems were used in the previous studies. In the previous studies, cylindrical [9] and half cylindrical [10] holes were used for the trap of cells. The asymmetrical hole might be suitable for trap than the symmetrical hole. The depths of the micro holes were between 0.002 mm and 0.01 mm in the previous studies [8−13]. The cell is sensitive to the height of micro ridge on the scaffold [14]. In the present study, the depth of the grooves is 0.0045 mm, which is smaller than the diameter of the cells. The deeper hole may have advantage to trap cells. At the shallower trap, on the other hand, trapping depends on the condition of each cell. The duration of the trapped time of the cell might relate to interaction between the micro hole and the cell: affinity between the cell and the surface of the micro pattern, or deformability of the cell.

The results of the present study show that the movement of cell travelling on the wall is modified by the oblique micro groove on the wall under the cell velocity lower than 1 mm/s. The angle of 45 degrees in the horizontal plane between the longitudinal direction of the groove and the flow direction is effective to shift the streamline of the cell. The shift movement along the oblique groove depends on the several parameters: the diameter of cells, the width of the groove, the velocity of the cell, and the kinds of cells. The cell density in the suspension is very low in this study to reduce the interaction between the cells. Cells might be sorted by the traveling length along the microgroove.

Reynolds number (*Re*) is calculated by

$$Re = \rho v d / \eta$$

In this formula, $\rho$ is density of the fluid [kg $m^{-3}$], $v$ is the circumferential velocity [m $s^{-1}$], $d$ is the distance [m] between the moving wall and the stationary wall, and $\eta$ is the viscosity of the fluid [Pa s]. *Re* is $3\times10^{-2}$, when $\rho$, $v$, $d$, and $\eta$ are $1\times10^{3}$ kg $m^{-3}$, $1\times10^{-3}$ m $s^{-1}$, $5\times10^{-5}$ m, and $1.5\times10^{-3}$ Pa s, respectively. The turbulent flow may not occur in the flow of the small value of Reynolds number.

## Conclusion

Oblique micro grooves have been designed to sort biological cells, which flow through a micro channel *in vitro*. The micro groove of a rectangular shape has been successfully fabricated on the surface of the polydimethylsiloxane plate with the photolithography technique. C2C12 (mouse myoblast) was used in the test. The results show that the cell is trapped by the micro grooves and that the traveling length along the groove is related to the diameter of the cell, to the width of the groove, and to the velocity of the cell. The designed microgrooves in micro-flow-channel has availability for cell sorting.

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