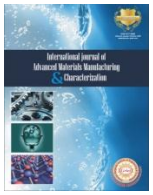

Advanced Materials
Manufacturing & Characterization

journal home page: www.ijammc-griet.com

Nano/Micro Fluidic Systems for Circulating Tumor Cells (CTCs) Rapid Detection and Diagnosis

Fan-Gang Tseng

Professor and Chairman, Engineering and System Science Department , Deputy Director, Biomedical Technology Research Center
National Tsing-HuaUniviersity, Taiwan.

ARTICLE INFO

Article history:
Received 10 Nov 2012
Accepted 26 Dec 2012

Keywords:
monolayer
hydrodynamic
shear-gradient.

ABSTRACT

Despite the recent advancement of biotechnology and pharmaceutical research, cancers remain the leading cause of human mortality. It is vital to diagnose cancers at an early stage when treatment can dramatically improve prognosis. So far, low-cost and easy to operate devices, which allow efficient isolation and sensitive detection of circulating tumor cells (CTCs) for routine blood screening, remain lacking. This talk will introduce a novel micro fluidic platform which can isolate CTCs from the real blood sample in 30 minutes: this system includes a high throughput blood cell separation chip which can separate white blood cells with CTCs from red blood cells and platelets by inertial and suction actions; a nano structured surface which can allow higher retention rate of CTCs on the surface for sample enrichment by 100 folds from 1/107 upto 1/105 CTCs/WBCs.and the enriched sample will go through a final cells self-assembly process into a densed monolayer on a cell assembly chip for in parallel inspection at high speed. As a result, the CTCs can be identified in 30 minutes by the integration of these three chips altogether. Isolated CTCs will still be in vital and can be further characterized and cultivated for the identification of cancer stem cells for prognosis

Introduction

Despite the recent advancement of biotechnology and pharmaceutical research, cancers remain the leading cause of human mortality. It is vital to diagnose cancers at an early stage when treatment can dramatically improve prognosis. So far, low-cost and easy to operate devices, which allow efficient isolation and sensitive detection of circulating tumor cells (CTCs) for routine blood screening, remain lacking. This talk will introduce a novel micro fluidic platform which can isolate CTCs from the real blood sample in 30 minutes: this system includes a high throughput blood cell separation chip which can separate white blood cells with CTCs from red blood cells and platelets by inertial and suction actions; a nano structured surface which can allow higher retention rate of CTCs on the surface for sample enrichment by 100 folds from $1/10^7$upto $1/10^5$ CTCs/WBCs. ; and the enriched sample will go through a final cells self-assembly process into a densed monolayer on a cell assembly chip for in parallel inspection at high speed. As a result, the CTCs can be identified in 30 minutes by the integration of these three chips altogether. Isolated CTCs will still be in vital and can be further characterized and cultivated for the identification of cancer stem cells for prognosis.

- Corresponding author: Dr. Fan-Gang Tseng
- E-mail address: fangang@ess.nthu.edu.tw
- Doi:http://dx.doi.org/10.11127/ijammc.2013.02.001

High Throughput Blood Cell Separation Chip

Recently, the inertial migration of particles in micro-scale flows has received much attention due to its promising applications, such as the pinched flow coupled with inertia microfluidic for circulating tumor cells (CTCs) separation [1,2]. In microfluidic chip the particles suspended in rectangular channels are known to be focused near the inner wall of each cross-section channel as the channel Reynolds number (Re) increases due to the lift force balance and the hydrodynamic interactions of the particles with the wall. The hydrodynamic have two major forces that made the particles migrate. One is the wall repulsion force due to the steric crowding effect between the particle and the wall, and another is the inertial lift force that originates from the shear-gradient of the Poiseuille's flow. The wall repulsion force pushes the particle away from the wall and the inertial lift force draws the particle toward the wall. Hence, the balance between these two oppositely directed forces induces an particles or cells equilibrate at a certain position [3]. And this talk proposes to employ these two forces together to separate whole blood cells into white and red blood cells in large volume, as shown in Fig. 1. [4]

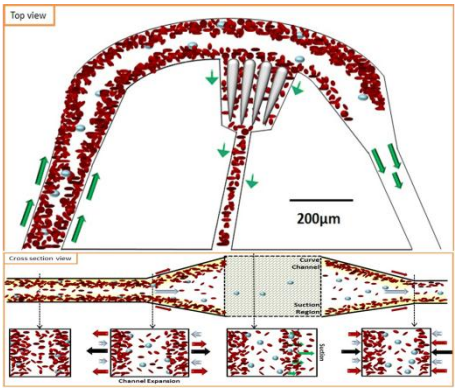

Fig. 1: Schematic of cells migration in confined main channel and absorption along inner-wall of curve channel. Particles in flow experience drag and lift forces yield the particles to equilibrium position as shown at channel cross section.

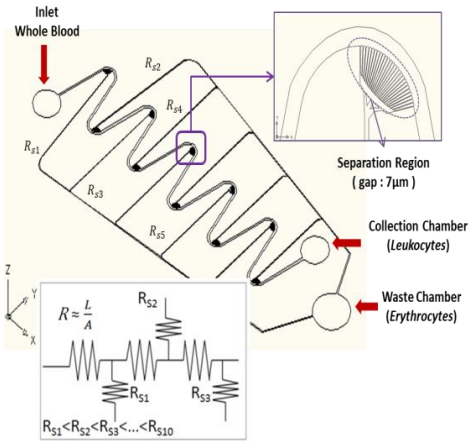

Fig. 2: Microfluidic channel geometry. 200μm(W) x 70μm (H) with symmetrically curve channels along main channel.

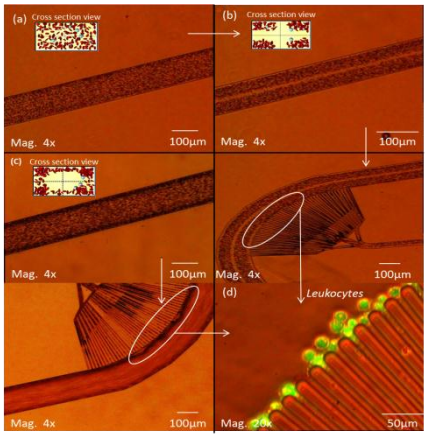

Fig. 3: (a) Whole blood cells at initial flow in channel. (b)Human blood cells with 20X buffer dilution begin to migrate at inner-wall by increasing the injection flow rate (1mL/min.). (c)Cells crowded by increasing the blood cells concentration with 5X of buffer dilution. (e)Leuthrocytes in the curve channel rejected at (~7μm) entrance of separation region.

The overall device design for the rapid blood cell separation is shown in Fig. 2, consisting of a serial curved flow channels with alternative curving directions, coupled with semi-circular array filters set with 7μm entrance gap and connected to a suction flow. The width of this gap is set to 7μm for deformable red blood cells (~7μm in diameter and thickness of 2μm) to be sucked out while keeping the leukocytes (> 10μm in diameter) rejected at the separation entrance. The blood was diluted with physiological saline in a ratio (volume) 1: 20 and 1: 5 before pumped into the chip.

The separation results were shown in fig. 3. After the separation, each output was taken and spread in a blood counting chamber for cell number counting. As a result ~82%separation efficiency can be achieved for 10 curves purification at the end of channel.

Cells Self-Assembly Chip

On the other hand, flow cytometry is the key technology that enables high-through put detection and isolation of specific cells; however, it is very time consuming and costly. As a result, two-dimensional(2D) cells arrays [5] were developed to reduce the cost of cells screenig and analysis. However, a 2D cells array consists of two contradictory difficulties: one is, a huge number of single cells must be close enough to reduce the observation area, but cannot stack multi layer aggregation to shield the fluorescence detection.

Traditional cells arrays usually need special structures to trap cells, not only cause more difficulties in operation but also limit the density of tissue culture. In this talk, we propose a simple, rapid and economic technique to form huge quantity of living cells into a self-assembled high density mono layer array for cell screening and tissue engineering with the employment of gravity a force and fluid force. [6]

The chip design for 2D specific cells idenfication is shown in Fig. 4, containing wells with diameter of 8mm and 1mm deep, and at the bottom the wells are connected to a very shallow slit with a height of 10μm and small outlets for liquid evaporizing to the atmosphere. Fig. 4 shows the operation principle. HL-60 cells in PBS were dropped into the well originally containing PBS, then cells gradually sink down to the chip bottom surface. Different from traditional cells spreading, this novel cells array chip provide a sidefluidforce pushing cells together with PBS out of the well because of evaporation of PBS from the small outlet described before,thus prventing cells stack into multiple layers.

Since the slit is only 10μm, thinner than the cells diameter, so the slit works as a filter to stop cells preading outward. Thus after 5 minutes a dense mono layer cells array can be formed from the center of the wells, as shown in Fig. 4(c).

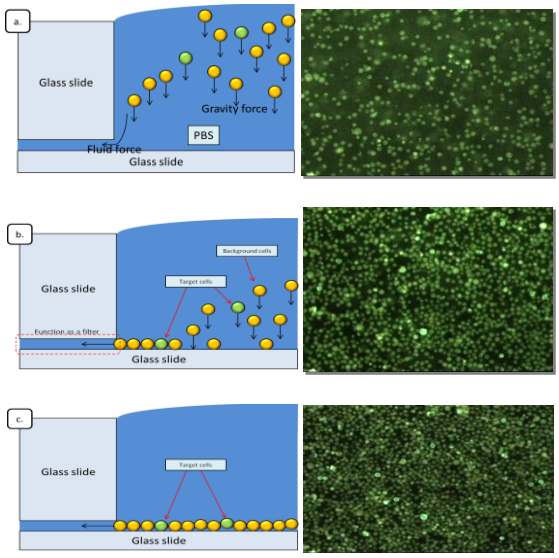

Fig. 4. Schematic cross-sectional view of the cells aligning process

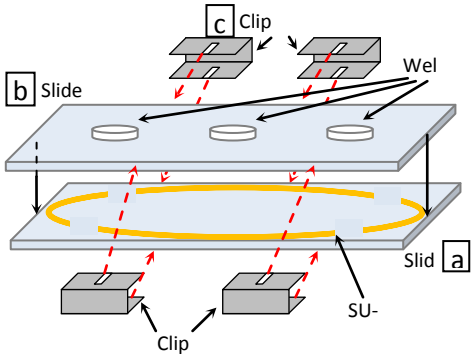

Fig. 5 System assembly schematic diagram

The fabrication process of the cells assembly chip is shown in Fig. 5, one slide glass with thin (10μm) SU-8 spacer (Fig. 5a) and several 8mm diameter through-holes (Fig. 5b) on the other. These two sub strates were then clamped with clips (Fig. 5c). Fig. 6 shows the cell screening test, the mixture of targeted cells with background cells at different ratios are tested by this chip. The individual targeted cells can be easily identified from the background cells arranged in 2D dense array. The lowest concentration this chip canprovide in the current experiment is 1/10,000, as shown in Fig. 6(d), opening up a new opportunity for rapid and in parallel targeted cells detection.

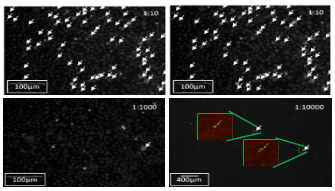

Fig. 6 Different ratio of targeted cells (Green) to background cells (Red)

References

1. Andrew W. Browne *et al., Lab Chip,* 2011, 11, 2440–2446
2. Jong Yoon Han *et al. Lab Chip*, 2011, 11, 1870
3. Dino Di Carlo, *Lab Chip*, 2009, 9, 3038–3046
4. H-C Tseng, R-G Wu,H-Y Chang,and F-G Tseng,*IEEE MEMS*, Seattle, USA, 2012.
5. Mordechai Deutsch et al., Lab Chip, 6, 995 (2006)
6. Y-C Chang, T-J Chen, and F-G Tseng, *MicroTAS*, Katanawa, Japan, 2012