Sensors and Actuators B 185 (2013) 245–251

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Cancer cell separator using size-dependent filtration in microfluidic chip

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ARTICLE INFO

Article history:
Received 24 October 2012
Received in revised form 12 February 2013
Accepted 8 April 2013
Available online xxx

Keywords:
Microfluidic chip
Cell sorter
Peritoneal washes
Gastric cancer
Enrichment

ABSTRACT

We have developed a microfluidic chip that can separate gastric cancer cells from peritoneal washes. Gastric cancer cells tend to be agglomerated in the peritoneal wash, and are larger than the other cells and the soft tissue fragments. This paper presents a novel microfluidic chip to enrich low-concentrated cancer cells from the peritoneal wash by size-dependent cell filtration. This microfluidic chip has the advantage that it can separate agglomerated cells in high speed because larger objects are filtered by a gap in the microchannel. Two-step exposure was performed to fabricate a precise uneven channel for cell filtration. The main and shallow channels have heights of 100 μm and 8 μm, respectively. Furthermore, inserting fins in the microchannel that assist sheath flow enhanced continuous and robust separation against flow fluctuation in the microchannel. The microfluidic channel was designed to have high filtration efficiency and a high throughput while having a compact profile. We used patient's peritoneal washes to isolate cancer cells. As a result, we were able to enrich the cancer cells in peritoneal wash.

1. Introduction

Gastric carcinoma is one of the leading causes of cancer death in Japan. Peritoneal carcinomatosis represents the most common route of tumor dissemination in patients with this disease, and recurrence is most likely caused by the presence in the peritoneal cavity of metastatic free cancer cells exfoliated from the serosal surfaces of primary cancers [1,2]. Peritoneal lavage cytology is commonly used to diagnose peritoneal carcinomatosis. However, the diagnostic accuracy of this method is not high enough to cure this disease. Even when the cancer cells are detected, it is difficult to determine the most effective therapy, especially the amount and type of anticancer agent. From this background, it is desperately required to detect and collect the low-concentrated cancer cells (0.001–10%) [1] in peritoneal washes and ascetic fluid with little damage on the cells. Moreover, by using a micro device to detect and collect the cells, it will be possible to diagnose peritoneal carcinomatosis at the patients' bedside.

Particles in a microfluidic chip can be separated by various techniques, including microfabricated fluorescence-activated cell sorting (FACS) [3,4], optical force switching sorting mammalian cells [5], magnetic-activated cell separation (MACS) [6,7], acoustophoresis particle separation [8,9], and dielectrophoresis (DEP) force separation [10–12]. However, these techniques have some problems of cost, size, and damage on the cells. FACS may damage cells during droplet generation and it requires a complex, expensive optical system to recognize different cells. In MACS, the magnetic matrix needs to be modified with a special antigen or antibody and it is difficult to accurately operate the modified magnetic matrix on a chip. The DEP method has been demonstrated as an effective and selective method in concentrating, manipulating, and separating cells and viruses that do not have a size difference simultaneously. However it requires strong polarization charges in a non-uniform electric field or sensitive flow control in a microchannel since DEP force is relatively small.

On the other hand, microfluidic chips have been used for cell separation. Separation methods based on microfluidic chips containing structures such as micro-pillars [13,14], micro-weirs [15], and cross-flow [16] with holes used to separate cells of different sizes cannot only be a simple and non-destructive method, but also permit easy integration of other different processes in a single device. However, most of the current separation microfluidic chips are susceptible to pressure fluctuation or flow fluctuation in the microchannel.

As typified by circulating tumor cell (CTC), a variety of analytical platforms have been developed based on microfluidic and filtration devices for capture of cancer cells from blood draw. However, the clinical significance to enriching gastric cells from peritoneal washes has not been fully verified [17–19]. In this paper, we present a novel microfluidic chip to enrich cancer cells that are present at ultra-low concentrations from peritoneal washes by size-dependent cell filtration that improves the robustness of the flow. Most free cancer cells tend to aggregate in the peritoneal washes so that they become larger than that of other cells

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http://dx.doi.org/10.1016/j.snb.2013.04.030

[20] such as blood cells, mesothelial cells, endothelial cells and fibroblasts. We demonstrate size-dependent cancer cell separation using a microfluidic chip that utilizes the principle of separation gap. The isolation of these larger cells from peritoneal washes will be challenging issue since typical studies require the continuous separation to process large volumes [21,22].

2. Material and methods

2.1. Design and theory of microfluidic chip

The proposed separation scheme employs an appropriately designed gap between bottom and ceiling of a microchannel to prevent the fluid flow pathway, thus allowing cells of defined sizes to be separated into the different flow channels (Fig. 1). The main channel and shallow channel have heights of 100 μm and 8 μm relative to the glass substrate, respectively. In peritoneal washes of gastric cancer patients, we can observe the cancer cell aggregates, the blood cells and the other cells, and those sizes are about 25–100 μm, 7 μm and 10 μm, respectively [23]. Most cells (i.e., those smaller than 8 μm) were separated within the shallow separation region which was located on the left-hand side of the main channel by sheath flow considering cell deformation. Sheath flow was utilized to collect cells along the separation wall of the channel prior to separation. The main channel had strategically placed diamond-shaped fins that altered the flow pathway. The adjacent fins were separated 300 μm and the lateral sides of the fins were parallel to the separation wall, respectively. Fig. 2 shows the simulation result of streamline in the microchannel. The remarkable streamline towards the separation wall without a fin will squash the cell more than gap height, and will pass these cells to the shallow channel. The vertical force with fin was decreased by an average of 70%. The strategic placement of the fins causes sheath flow to generate a vertical force on the separation wall that minimizes the normal displacement of the cell caused by the inertial forces of the cells. The fins contributed to disrupt the sheath focusing. Consequently, the cells are efficiently guided to the shallow separation region.

Moreover, this separation is continuous and robust against flow fluctuations in the microchannels because the separation is performed mechanically by the separation wall along the microchannel. Fig. 3 shows the evaluation of the streamline as a function of flow velocity by finite element method (FEM) analysis (COMSOL Multiphysics 4.1, COMSOL Co., Ltd.). In this analysis, the streamline is not affected by flow fluctuation of the sheath flow between 0.1 mL/h and 10.0 mL/h. Other inlet A or outlet A and B were given the flow rates of 1.0 mL/h. The design of cell filtration is expected to give a good robustness against pressure fluctuation and flow fluctuation.

2.2. Fabrication of microfluidic chip

A microfluidic chip consists of a polydimethylsiloxane (PDMS) microchannel and a glass substrate. Fig. 4a depicts the processes

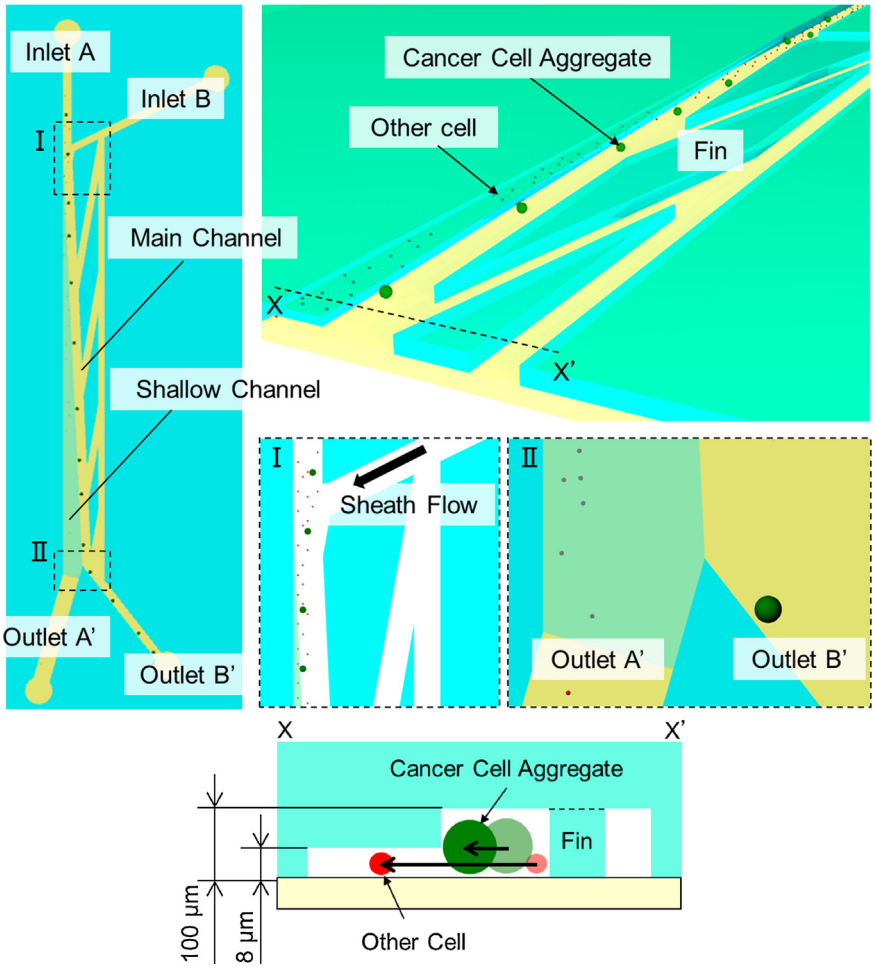

Fig. 1. Concepts of the microfluidic chip for filtering ultra-low concentrated cancer cells in patient's peritoneal washes. The proposed separation scheme employs appropriately designed gap between bottom and ceiling of a microchannel to prevent the fluid flow pathway, thus allowing cells of defined sizes to be separated into the different flow channels.