GENTLE RETENTION OF CELLS IN A FOCUSING ULTRASONIC RESONATOR INTEGRATED IN A CHIP-BASED PERFUSION SYSTEM FOR CELL CHARACTERIZATION AND ON-CHIP CULTIVATION

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ABSTRACT

Inside a microfluidic chip, lateral acoustic forces are obtained in an expansion chamber which focuses the sound field originating from an external transducer with a refractive element operating at 7 MHz. The chip system is designed for pre-alignment of cells in a straight inlet channel, followed by positioning and retention of cells in the expansion chamber. Trapped cells may be characterized using any kind of high-NA optical microscopy. Here, we demonstrate the use of conventional light transmission, phase contrast, dark-field and fluorescent microscopy imaging. The goal is to use the present device as a micro-bioreactor combined with optical characterization of individual cells.

Keywords: Acoustic, cell handling, focusing resonator, optical microscopy

1. INTRODUCTION

Ultrasonic standing wave (USW) technology is a promising tool for gentle cell handling in microfluidic chips [1]. Most USW chips utilize straight microchannels, in order to produce a plane standing wave between the channel walls [2-4]. While such systems primarily are efficient for continuous particle separation, retention systems require a lateral force component parallel to the fluid flow. For example, lateral forces can be obtained by a miniature transducer integrated in the microchannel, producing strong near-field gradients in the flow direction [5, 6]. In the present paper, we suggest an approach where lateral forces are obtained in an expansion chamber which focuses the sound field in the flow direction. The system is designed for pre-alignment in a straight inlet channel, followed by positioning and retention of cells in the expansion chamber. The purpose of the device is to serve as a miniature perfusion system for individual characterization of cells and on-chip cultivation.

2. EXPERIMENTAL ARRANGEMENT

The chip system, depicted in Fig. 1, consists of a dry-etched silicon structure sandwiched between two glass layers, and an external transducer with a refractive element placed on top of the chip for efficient coupling of ultrasound into the channel. By the use of single-frequency (~7 MHz) ultrasonic actuation, several manipulation functions are achieved simultaneously at different positions inside the chip depending on the channel geometry.

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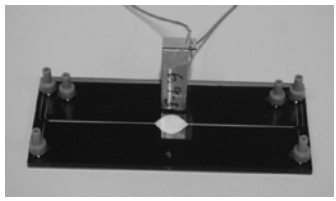

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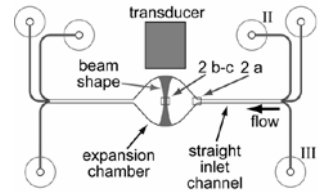

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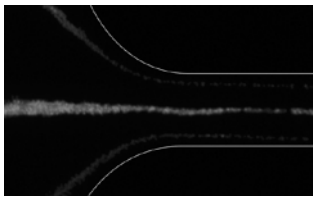
Figure 1. Photograph (a) and schematic (b) of the chip design featuring an expansion chamber actuated by the ultrasound transducer. The areas marked (2 a) and (2 b-c) are the imaged regions in Fig. 2. Injection of cells can be made in one or several of the inlet holes marked I, II and III, matching the three focused lines in Fig. 2 a.

In the straight inlet channel, manipulation in two dimensions is obtained by superposition of vertical and horizontal standing waves, both perpendicular to the flow, resulting in levitation and formation of three lines of cells, in the laminar flow from the corresponding inlet. In the expansion chamber, the curved reflecting walls create a focused horizontal standing-wave field superposed on the vertical levitating field, resulting in positioning and retention of cells in compact monolayer aggregates during perfusion of cell medium (see Fig. 3a). The USW modes in the expansion chamber are depicted in Fig. 2, where 10 µm polystyrene beads are used to visualize the pressure node pattern. Besides the focusing ability for generation of stronger lateral forces, the expansion chamber also has the advantage of reducing the flow velocity which in turn reduces the competing fluid drag force. Furthermore, the superposed fields guide the cells through the chip without any unwanted surface contact with the channel walls.



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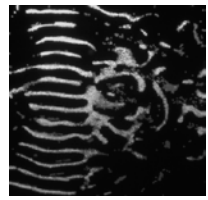

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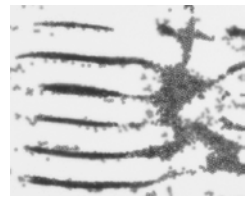

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Figure 2. Visualization of the node pattern in the expansion chamber using 10 µm fluorescent beads with node-to-node distance ~100 µm. (a): Levitation and formation of three lines, picture shows the transition region between the straight inlet channel and the expansion chamber. The channel walls are outlined in white. (b-c): Pattern of retained beads at the center of the expansion chamber. In all images, the flow direction is from right to left.

3. CELL CHARACTERIZATION

Cells trapped in the expansion chamber may be studied and characterized using any kind of high-NA optical microscopy. In Fig. 3 and 4, we demonstrate the use of conventional light transmission, phase contrast, dark-field and fluorescent microscopy imaging of COS7E cells stained with the fluorescent viability indicator Calcein AM.

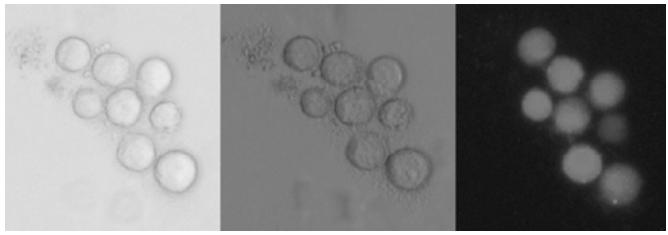

Figure 3. Positioned and retained COS7E cells (~10 μm) during perfusion of cell medium, visualized by transmitted light, phase contrast, and fluorescence microscopy (objective: 20X / N.A. 0.3). In all images, the flow direction is from right to left.

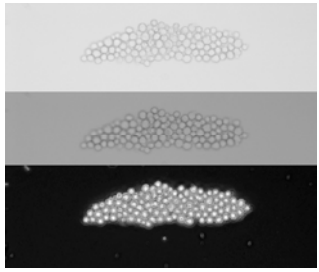

Figure 4. Positioned and retained COS7E cells (~10 μm) during perfusion of cell medium, visualized by transmitted light, phase contrast, and dark-field microscopy (objective: 20X / N.A. 0.3). In all images, the flow direction is from right to left.

When aiming for long-term (~days) cell retention and on-chip cultivation, it is important to maintain the biocompatibility of the system. Previous results include viability measurements and temperature control [1, 7]. In the present chip, the different microscopy techniques (cf. Fig 3 and 4) makes it possible to perform detailed optical characterization of cells without the need for toxic fluorescent labels. In the future, our device can be used as a micro-bioreactor with the option of individual cell monitoring and optical characterization.

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