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A cell delivery and pre-positioning system utilizing microfluidic devices for dual-beam optical trap-and-stretch☆

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ABSTRACT

This study reports a new microfluidic chip capable of delivering and pre-positioning cells in a pre-defined trapping zone, and followed by manipulation of buried optical fibers for on-chip, dual-beam, optical trapping and stretching. In this microfluidic system, microchannels, micropumps, microvalves, dielectrophoretic (DEP) electrodes and active fiber manipulators were fabricated and integrated using micro-electro-mechanical-systems technology to perform several crucial functions including transportation, pre-positioning and manipulation of cells. Experimental results showed that by integrating three micropumps connected in series, the cell samples were automatically delivered into the flow focusing area and then transported to the trapping zone. A single cell can be confined by microvalves and then elevated towards the optical trapping zone by a negative-DEP force operated at a low voltage ($20\ V_{p-p}$) and at a specific frequency (900 kHz). The active fiber manipulators can be used for optical trapping, manipulation, and stretching. A red blood cell was successfully trapped and stretched by a dual-beam, optical trap using the proposed microfluidic system. The developed system is promising for further applications that require trapping, manipulation and biomechanical analysis of a single cell or particle.

1. Introduction

Optical micromanipulation is now established as a powerful tool in many biological applications and colloidal science after the pioneering work by Ashkin on the trapping of particles with light [1]. Furthermore, optical tweezers have been widely investigated after the development of optical trapping with a single laser beam as a non-invasive manipulation technique for microparticles such as biological cells and microspheres [2]. It has also been demonstrated as a promising approach in a variety of applications such as cell biosensors, single cell molecular biology research, and laser-assisted, in vitro fertilization [3–7]. Several advantages of the optical manipulation technique can be observed including the ease of manipulation of a single cell to specific locations and the ability to perform manipulation in a closed, sterile environment without danger of contamination. Optical tweezers technology has been employed for the precise manipulation of living cells and organelles within cells [8–10], and bio-molecules such as DNA and RNA [11]. However, the efficiency of optical tweezers can be degraded in a turbid biological environment since it is hard to precisely focus a laser beam which is essential for optical trapping.

In addition to single-beam optical traps, dual-beam optical stretchers consisting of two counter-propagating laser beams from two well-aligned single-mode fibers (SMF) have been developed for non-invasive and non-contact trapping and stretching of biological cells to measure their viscoelastic properties [12]. Advantages of this fiber-optical, dual-beam trap include better mechanical stability, and the ease of changing trapping laser sources emitting from the pigtailed fibers [13]. Furthermore, an integrated and compact system without complicated and bulky optical components is feasible since the fiber-optical implementation is simple, robust and inexpensive. Hence, the integration of a dual-beam optical trap and microfluidic systems for biological detection and analysis using micro-electro-mechanical-systems (MEMS) techniques has been extensively investigated in the past few decades [14–16]. In order to improve the coupling efficiency of the head-on single-mode fibers, micro-scale active couplers including piezoelectric [17], electrostatic [18], electrothermal [19,20] and thermal actuators [21] fabricated by MEMS processes have been reported to reduce the insertion loss due to the extremely small core of the single-mode

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Nomenclature

AC	alternating current
CCD	charge-coupled device
DEP	dielectrophoretic
EMV	electromagnetic valve
IR	infrared
ITO	indium-tin-oxide
LOC	lab-on-a-chip
MEMS	micro-electro-mechanical-systems
PDMS	polydimethylsiloxane
PR	photoresist
RBC	red blood cell
SEM	scanning electron microscope
Si	silicon
SMF	single-mode fibers
UV	ultra-violet

optical fibers. However, the relatively high cost and complicated fabrication processes still hinder their practical applications when integrated with microfluidic systems. Our group has previously reported an easy and reliable active coupler for co-axial fiber alignment utilizing air-driven, controllable moving walls capable of on-line optical detection or manipulation [22]. The position of the single-mode fibers can be actively adjusted in two-dimensions to improve the coupling efficiency and to reduce the insertion loss with a simple fabrication process, so that these prototype devices can be integrated onto a microfluidic chip system successfully.

Recently, MEMS technologies based on the principles of biology, analytical chemistry and using miniaturization techniques have proven to be a promising approach to achieve the concept usually referred to as "lab-on-a-chip" (LOC). A LOC system can perform sample analysis and characterization such as sample preparation, handling, maintaining optimum reaction temperatures, mixing, and sample separation/detection by integrating several critical miniature components. Traditionally, delivery and transport of cells or bio-molecules still remain an issue in a conventional dual-beam optical trapping system. Most of previous optical trapping systems are operated and manipulate cell samples under a stagnant flow. In order to increase the throughput for cell manipulation, large-scale pumping devices such as syringe pumps are usually used to transport samples in a continuous flow. Hence, a microfluidic system integrated with microfluidic components such as micropumps can be utilized for automatic, continuous sample delivery, which is essential to realize higher throughput and continuous sample manipulation and analysis without extra bulky instruments.

Microfluidic devices for handling a minute amount of fluids have been widely investigated since micropumps structures were first demonstrated in the early 1980s [23]. Various membrane-actuation methods including reciprocating displacement of piezoelectric materials [24], electrostatic [25], thermopneumatic [26,27], electromagnetic [28] and many other activation methods have been demonstrated to realize different micropumping devices. Besides, an effective and reliable pneumatic micropumping device utilized to handle fluids in microchannels can be easily integrated onto bio-analysis chips [29]. A pneumatically driven microvalve structure capable of on-chip sample flow manipulation in a microfluidic system was developed [30]. A microvalve structure can block the microchannel and stop the sample flow accordingly. Additionally, dielectrophoretic forces have been reported as an approach to manipulate a polarizable particle/cell under a non-uniform alternating current (AC) electrical field [31]. The DEP forces have been demonstrated as a promising approach capable of various types of cell manipulations [32]. The feasibility of DEP separation and trapping of sub-micrometer-scale bio-particles has also been reported [33].

In this study, we have developed a new microfluidic system capable of actively aligning the buried optical fibers and continuously delivering/pre-positioning cells in an operating area for on-chip dual-beam optical trapping applications. The proposed microfluidic system integrated with several microfluidic components including microchannels, micropumps, microvalves, dielectrophoretic electrodes and active fiber manipulators, which were fabricated using MEMS technology to perform several crucial functions for dual-beam optical trap-and-stretch stably and efficiency, including cells transportation, separation, pre-positioning, and alignment of buried single-mode fibers. The fiber coupling devices composed of a pair of active fiber manipulators can align the optical fibers efficiently for dual-beam trap. Only one air inlet and electromagnetic valve (EMV) are required to activate the fluid transport system composed of three connected pneumatic micropumps to generate flow focusing effect. The sample cells can be focused and transported to a trapped area orderly utilizing the fluid transport system. The single cell can be confined in the trapped area by a pair of microvalves and levitated to the same level with laser axes by using negative-DEP force and a design involving flow channel and fiber channels with different depths. By using the sample pre-positioning mechanism, the cell trapping efficiency can be improved. Consequently, an automatic optical trapping system with functions including high-throughput cell sample transportation, pre-positioning of the cell sample, and active optical fiber manipulation can be realized by this integrated microfluidic chip device.

2. Experimental

2.1. Design

The major contribution of this study is the new design of a microfluidic chip capable of performing transportation and pre-positioning of cells in a specified area utilizing micropumps, microvalves and dielectrophoresis forces with on-chip dual-beam optical trapping and stretching. Fig. 1(a) shows that the proposed microfluidic system is composed of three major regions including a sample transportation area, a flow focusing area and an optical manipulation area. The micro-scale components are composed of flow channels, air chambers, optical fiber channels and indium-tin-oxide (ITO) electrodes. In order to deliver the cell samples inside the flow channels in an orderly manner, sample and buffer flows were first transported through the microchannel to generate a hydrodynamic focusing effect in the flow focusing area by using three pneumatic micropumps. The pneumatic micropump consists of three air chambers with different volumes and an underlying microsample flow channel [29]. The resilient polydimethylsiloxane (PDMS) membranes between the air chambers and the underlying flow channel can be deformed sequentially when the compressed air is injected into the air chambers, and the time-phased deflection among membranes causes peristaltic motion to transport the liquid forward. The velocity of the sample flow was determined by the operating frequency of the air injection, the applied air pressure and the dimensions of the membranes [34]. Accordingly, the cell samples in the central flow channel can be focused into a narrow stream by the neighboring buffer flows (sheath flows) with higher flow velocity. The relative sheath-to-sample flow ratios can be used to control the width of the focused stream [35,36]. In this study, in order to generate different flow velocities for the sample flow and the sheath flows, the widths of the sample flow and the sheath flow channels were designed with different widths (100 and