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Micromagnetic Cancer Cell Immobilization and Release for Real-Time Single Cell Analysis

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ABSTRACT

Understanding the interaction of live cells with macromolecules is crucial for designing efficient therapies. Considering the functional heterogeneity found in cancer cells, real-time single cell analysis is necessary to characterize responses. In this study, we have designed and fabricated a microfluidic channel with patterned micromagnets which can temporarily immobilize the cells during analysis and release them after measurements. The microchannel is composed of plain coverslip top and bottom panels to facilitate easy microscopic observation and undisturbed application of analytes to the cells. Cells labeled with functionalized magnetic beads were immobilized in the device with an efficiency of 90.8 ± 3.6%. Since the micromagnets are made of soft magnetic material (Ni), they released cells when external magnetic field was turned off from the channel. This allows the reuse of the channel for a new sample. As a model drug analysis, the immobilized breast cancer cells (MCF7) were exposed to fluorescent lipid nanoparticles and association and dissociation were measured through fluorescence analysis. Two concentrations of nanoparticles, 0.06 μg/ml and 0.08 μg/ml were tested and time lapse images were recorded and analyzed. The microfluidic device was able to provide a microenvironment for sample analysis, making it an efficient platform for real-time analysis.

1. Introduction

Real time data recording is important for studying live cells. Live cell studies can provide detailed information about cell-cell interaction, receptor-ligand interaction or downstream cellular pathways [1–3]. Studies such as real time apoptosis, cell-substrate interaction have been reported [4,5]. Live cell visualization and data recording often require immunofluorescent markers or nanoparticles such as lipid micelles. These techniques have been employed to study cancer cell signaling pathways, metastasis and cancer targeting in vivo [6,7]. Studies have shown cancer cell functional heterogeneity in a tumor and it is important to study single cells in real time [8,9]. Single cell analysis of cancer cell would give an understanding of cancer initiation, progression and metastasis as well as cellular signaling pathways, which allow us to understand their therapeutic responses [10,11]. Drug screening studies done on single cells can also help target cells more specifically by knocking down some signaling pathways [12].

Single cancer cell studies have been conducted on microfluidic devices or 'Lab on a chip' devices to provide a platform to create the microenvironment needed to study cellular behavior in a dynamic system [13–16]. These devices were used for various biological assays where sample volumes needed for analysis should be minimized [17]. Important requirements for such devices are that they do not disturb cell-analyte interaction or change cell responses to analytes while immobilizing them in the channel efficiently.

Techniques that have been used to trap single cells in a microfluidic channel include dielectrophoresis (DEP), Polydimethylsiloxane (PDMS) micro traps, microfilteration and immunomagnetic capture [18–21]. Dielectrophoresis can selectively capture cells for applications such as cell fusion studies [22]. PDMS traps in a microfluidic channel can easily immobilize cells for on-chip single cell culture [23]. Physical attributes such as size and stiffness are also used for capturing cancer cells using microfiltration [24]. Even though these techniques have successfully demonstrated the ability to capture cells, there are few drawbacks: PDMS traps disturb uniform flow in the channel and may absorb molecules or nanoparticles under testing [25], and electric fields used in dielectrophoresis may alter cellular activities. Immunomagnetic capture for cell separation [26,27], culture [28],

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sorting [29–31] and sifting [32] on a microfluidic platform has been demonstrated. The inclusion of micron sized iron-PDMS posts [33] and pillars [34,35] within microchannel have been studied. Magnets have been integrated within the microfluidic channel as stripes and toothed pattern [36] from paramagnetic [37] and ferromagnetic materials [38], magnetic beads [39] and electroplated metal stripes [40]. In order to magnetize these patterns within the microchannel, an external magnetic field source is applied such as permanent magnet [26] and solenoids [41,42]. These external magnetic field sources either surround the channel or are placed directly on top or bottom of the channel. This makes the device less compatible with commercially available upright or inverted microscopes for long term real time cell analysis. Here we aim to design a simple microfluidic device for effective immunomagnetic cell capture and undisturbed serial sample analysis in a single device in the following way:

1. An array of thin micromagnets was built through minimal fabrication steps to achieve easy cell immobilization. A flat C-shaped solenoid designed to fit most microscopes was used to activate the soft micromagnets and made the process of immunomagnetic immobilization reversible.
2. The use of standard plain coverslips as top and bottom panels, made the microfluidic device simple and useful for general live cell on-chip analysis under either an inverted or upright microscope. The design facilitates addition of reagents under a uniform laminar flow to study captured single cells.

In order to assess real time live cell analysis, we use lipid bilayer nanoparticles as a model drug and quantify cell-nanoparticle interaction [43]. An application of this device is studied using fluorescent nanoparticles (liposomes) that are a potential drug delivery vehicle [44,45]. This analysis provide quantitative information useful in drug testing on rare cancer cells. Nanoparticles being a model for drug delivery, their interaction with cells is of importance in a microfluidic channel as their delivery into the cells can be studied in detail with this device.

2. Materials and methods

2.1. Microchannel fabrication

The schematic in Fig. 1 shows the device design. The main objective is to design a microfluidic device which immobilizes cells without causing additional stress to the cells with high efficiency and enables real time analysis on-chip. The microfluidic device consists of a microfluidic channel, patterned nickel as the micromagnets and an external solenoid for active magnetization of micromagnets. The nickel patterns are formed on the bottom substrate to locally enhance the magnetic field generated by the external solenoid. The thin PDMS film with a cut-through pattern of the channel was plasma bonded on the bottom glass substrate such that the nickel pattern was aligned within the channel. Another glass coverslip was plasma bonded on top of the film to seal the channel. Inlet and outlets were made using PDMS blocks. The device was placed between the arms of a C-shaped solenoid. The solenoid produced an external magnetic field that in turn magnetized the nickel pattern. Nickel being a soft magnetic material, was able to magnetize and demagnetize with application or removal of external magnetic field. Nickel micro-pattern, acting as small magnets or micromagnets, enhanced the magnetic field to immobilize cells on the channel. The trapezoid geometry of the solenoid arms was designed to create a focused magnetic field that exerted an addition magnetic force on cells to be pulled towards the nickel pattern.

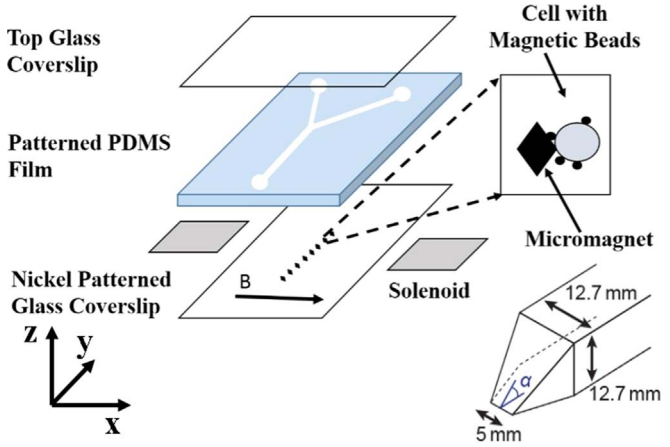

Fig. 1. Schematic of the cancer cell immobilization device with microfluidic channel patterned on a thin PDMS film and enclosed between two coverslips.

For nickel magnets, conventional photolithography using a positive photoresist (Microposit S1805) was used to pattern thermally deposited nickel (Kurt J. Lesker, PA, USA) on a glass coverslip as seen in Fig. 2A. Nickel thin film was wet-etched to obtain the micromagnet array (200–250 nm height) of 1000 micromagnets in a single channel. Each micromagnet (Fig. 2, inset) is in a shape of a diamond with an edge size of 64 μm. In order to make a microscope friendly channel that could be used with inverted as well as upright microscopes, thin film channels were designed using PDMS (Dow Corning Sylgard 184, Fisher Scientific) films. The Y shaped channel (0.024×1 mm) was cut on thin PDMS films (thickness:160 and 240 μm) using an electronic design cutting machine (Silhouette Portrait, Silhouette, Utah, USA). The use of the portrait cutter eliminates soft lithography steps that are usually used in conventional microfabrication of PDMS channels. Before the channel was covered with the glass coverslip, the channel was treated with Sigmacote (Sigma Aldrich) to make the surface hydrophobic to prevent cell attachment to glass coverslip. The C-shaped solenoid with 1600 turns, 21Ω, was developed using low carbon steel bars (0.5×0.5 in. cross section, McMaster Carr, USA). Solenoid arms facing the channel were machined at an edge angle α (see discussion in Section 2.2) to create a 5 mm-wide edge to create focused external magnetic field along the channel. The actual solenoid characterization was done using a Hall Effect sensor (SS490, Honeywell, USA) attached to a mechanical stage located at the center of the solenoid arms.

2.2. Magnetic field analysis

Magnetic separation has been well studied for detection of circulating tumor cells [27,46]. Theory behind magnetization of a soft magnet under an external magnetic field has been described in detail [47]. Briefly, when an external magnetic field H_{ext} is applied, the magnetic dipole of a soft magnetic material align to produce a net magnetization.

Magnetic force (F_m) that acts on a magnetic bead can be calculated as

$$F_m = \frac{N_{bead} \cdot V \cdot \Delta\chi_{bead}}{2\mu_0} \nabla B^2 \ldots \quad (1)$$

Here, $\mu_0 = 4\pi \times 10^{-7}$, is the magnetic permeability of vacuum, B is the magnetic field intensity, N_{bead} is the number of beads attached to cells, V and $\Delta\chi_{bead}$ are the volume and effective magnetic susceptibility of the magnetic bead, respectively [48]. We used the value of $\Delta\chi_{bead}$=0.65, according to Sinha et al. [48].

COMSOL Multiphysics (version 4.4), AC/DC module was used to simulate the magnetic field produced by the solenoid. The magnetic field generated by solenoid arms with edge angles of α=25°, 45° and 60° was simulated for comparison. Fig. 2B shows the magnetic force acting on a single bead (see Eq. (1)) in z direction plotted for −6 mm < z < 6 mm, considering the edges of the arms to be z=0. As shown in