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Ultrafast laser microfabrication of a trapping device for colorectal cancer cells

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ABSTRACT

Although celltrapping devices have been microfabricated and widely used for manipulation of bio cells, devices that trap multiple cells simultaneously are difficult to design because fabrication is complicated and time consuming. We designed and manufactured a microfluidic device using a polycarbonate plate with a flatness less than 200 μm and a gelatin-coated polyethylene terephthalate (PET) membrane. The device was used to capture colorectal cancer cells from one of the most common types of human malignant tumors. Microfluidic channels for the device were micromachined in minutes using a Computerized Numerically Controlled (CNC) engraving machine. We microfabricated multiple microholes on the PET membrane, which had a thickness of 13 μm, using an ultrafast, 1025 nm diode-pumped solid state femtosecond laser. The 100 microholes were drilled by spirally moving spot size of 4 μm laser beam. It is very important to obtain smooth and clean surface to avoid cell damages when they are trapped on the device. The relationship between the diameter changes of the microholes and variations in laser output power as well as laser fluence were investigated through parametric analysis. The average diameter of the holes increased exponentially with laser power. The gelatin-coated PET membrane was attached to the polycarbonate device and a syringe with a tube controlled negative pressure inside the channels of the cell-trapping device. Maintaining negative pressure inside the channels under the microholes on the PET membrane, colorectal cancer cells were dropped using the cell dropping pipette and successfully captured for manipulation under same environmental condition.

1. Introduction

Cell manipulation in microfluidic platforms can be applied to biomedical areas such as genomic mapping [1], oncology [2] and stem cell research [3]. Conventional cell studies are usually conducted with large population of cells, but this approach can lead to misunderstandings about cellular events. Therefore, studies on single-cell scale are necessary to obtain meaningful data [4].

A variety of methods are available for individual cell trapping [5] and can be classified as cell immobilization or contactless cell trapping. Hydrodynamic [6,7] or chemically induced cell trapping is a type of immobilization. Contactless cell trapping includes use of lasers, acoustics, dielectrophoresis [8–12] and magnetic trapping [13,14]. These devices have limited cell-capturing ability, depending on the cell type [13,15]. We focused on cell-capturing devices that use microfluidic channels. Studies on microfluidics published in recent years have described the underlying mechanisms of devices and their performances [16–18] Recently, femtosecond lasers, a microfabrication tool, have been used in cell-trapping devices [19,20]. Microholes can be fabricated in polyethylene terephthalate (PET) membranes without thermal damage because the pulse length of ultrafast lasers is less than 400 femtoseconds [21,22]. Since the cells are so delicate, rough surface of the holes on the trapping device may cause cell damages. Although some researchers fabricated cell trapping device using femto-second lasers, they failed to achieve very clean and smooth surface on the capturing holes [23].

Colorectal cancer (CRC) is one of the most common human malignant tumors with an age-adjusted incidence of 46.3 per 100,000 [24]. In the United States, CRC is the 4th common tumor after lung, prostate and breast cancers and 2nd common cause of cancer death after lung cancer [25]. Despite efforts of clinicians to find effective therapies against colorectal adenocarcinoma, which is the leading

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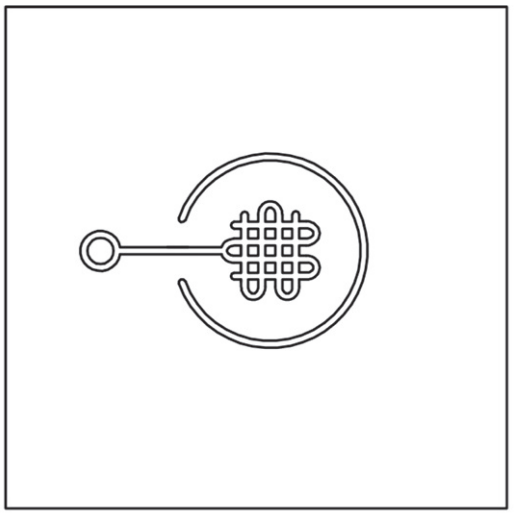
cause of cancer-related mortality, its incidence and associated death rates continue to rise rapidly. According to molecular biology and pathology, CRC is developed from normal epithelial cells but histopathological and molecular properties are different from them. The risk factors for CRC are age, polyps, sedentary lifestyle, diet, obesity, family history of CRC, and Inflammatory Bowel Disease (IBD). Although surgical resection is only curative treatment, early detection and screening is of crucial importance. As the case stands, likelihood of cure is greater when disease is detected at early stage. The general methods to detect the colorectal tumor are Fecal Occult Blood Test (FOBT), flexible sigmoidoscopy and standard colonoscopy. However, these screening methods have disadvantages respectively. The FOBT and flexible sigmoidoscopy can not detect some polyps and cancers, and abnormal growths in the upper part of the colon will be missed. In addition, the standard (or optical) colonoscopy test is highly sensitive but it still may not detect all small polyps, nonpolypoid lesions, or cancers. Because the CRC is the leading cause of death and early stages can be detectable, the screening is very important for prevention of CRC. The CRC screening method using the cell trapping device is a novel approach for colorectal cancer detection and one of promising and non-invasive tool to detect Circulating Tumor Cells (CTCs) from small blood of patient. We designed a new cell trapping device to enhance the accuracy of diagnosis for early stage of colorectal cancer.

In this study, we made spot size of 4 μm beam using femtosecond laser and drilled spirally from the center to side very carefully on precise motorized stage. As a result, we fabricated very smooth and clean surface on the multiple holes for singular cell-capturing device which is not reported in former studies to manipulate CRC cells without damages. We fabricated aligned holes on gelatin-coated PET membrane filters using ultrafast femtosecond laser. Microfluidic channels were engraved on the polycarbonate to create negative pressure between the microfluidic device and the gelatin-coated PET membrane. CRC cells were captured by the smooth and clean membrane holes. We applied femtosecond lasers for a cell-capturing device and manufactured a reliable microfluidic device.

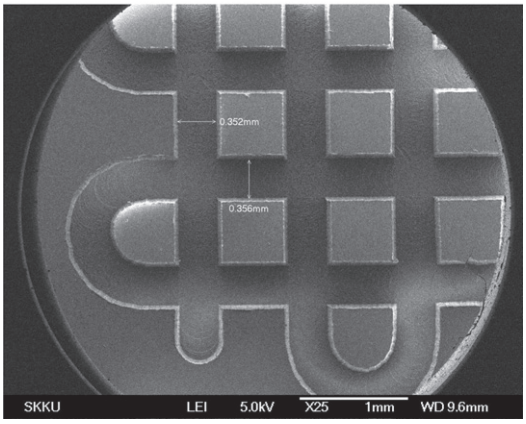
2. Experiments

A device was designed and microfabricated to trap colorectal cancer cells. The device consisted of two components: a microfluidic device and a membrane filter. A polycarbonate plate was used to micromachine the microfluidic device, which was the device base. An AutoCAD design for the device is shown in Fig. 1(a). The microfluidic channels were prepared to maintain negative pressure for cell capture and a round hole on the left side was connected to the channels to create pressure. A CNC engraving machine was used to micromachine the polycarbonate microfluidic device. The movement speed of the machining tool was 300 mm/min with 15,000 rpm. The engraving depth was 500 μm and channel width was 350 μm. A scanning electron microscope (SEM) image for the CNC engraved channels is presented in Fig. 1(b). The drilled holes on the membrane filter will be aligned at the center of the cross area on the device for cell capturing.

A 1025 nm wavelength diode-pumped solid-state femtosecond laser was used to fabricate microholes on the membrane filter that was attached to the microfluidic device. The material for the filter was a SPI-Pore™ gelatin-coated PET membrane with a thickness of 13 μm. The gelatin coating protected cells held on the filter surface by negative pressure. Moreover, very smooth and clean surface are important to avoid cell damages from the negative pressures. The schematics of the femtosecond laser and the microfabrication system are shown in Fig. 2. Focusing lens, mirrors and attenuator are used for precise focusing and laser beam delivery. A JenLas D2.fs femtosecond laser (JENOPTIK) was prepared and equipped on the precise motorized stage. The laser had an average output



(a)



(b)

Fig. 1. (a) AutoCAD design for microfluidic device (b) SEM image of CNC engraved channel on the device.

power of 4 W and wavelength of 1025 nm. Pulse width was 380 femtoseconds and maximum pulse energy was 38 μJ at a pulse repletion rate of 100 kHz. Laser and beam specifications are shown in Table 1. The TEM00 beam mode was used for the fabrication. The laser beam was delivered through an attenuator and mirrors and focused by focusing lenses. The energy of the laser was controlled by the motorized attenuator between mirrors 3 and 4. Laser spot size of 4 μm and precise motorized stage were used to make

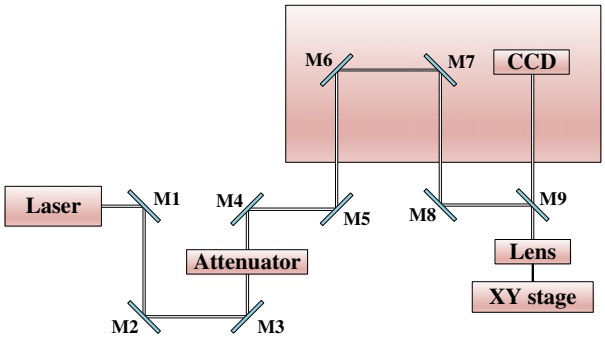


Fig. 2. Schematics of femtosecond laser microfabrication system.