Alignment of E. coli Bacteria in a PDMS Microfluidic Channel Using Ultrasonic Standing Wave

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ABSTRACT— Ultrasonic radiation force caused by ultrasonic standing wave has the capability to align microparticles suspended in fluid medium in its nodal or antinodal planes depending on the mechanical properties of particles and fluid medium. In some of the recent works, alignment of live cells like Cyanobacteria etc. inside a biocompatible Polydimethylsiloxane (PDMS) microfluidic channel were successfully carried out by utilizing the ultrasonic radiation force. This study is an extended work of these types of ultrasonic live cell manipulation in PDMS microfluidic channel. In this work, we demonstrate the alignment of E.coli bacteria, which is widely used in biotechnological research area, using ultrasonic radiation force in a PDMS microfluidic channel. With this successful alignment of E.coli bacteria it is proved that ultrasonic standing wave has the vast range of manipulation capability of different types of live cells.

Keywords— ultrasonic standing wave, live cell manipulation, PDMS microfluidic channel, e.coli bacteria.

1. INTRODUCTION

Microparticle manipulation in the fluid medium is essential process in the field of bioscience & biotechnology. Various types of microparticle manipulation techniques such as optical trapping [1-4], magnetophoresis [5-6], dielectrophoresis [8], acoustophoresis [9-16] etc have been investigate actively. Among those manipulation techniques, ultrasonic method is pioneer for different types of microparticle and live cell manipulations because of it non-contact and flow-through manipulation process. In a typical ultrasonic manipulation method, ultrasonic standing wave compels the particles to migrate towards either the pressure nodal or antinodal plane depending on the mechanical properties of particles and fluid medium. [17]

A very common ultrasonic manipulation method is the alignment of distributed particles suspended in a fluid flow in the nodal plane of ultrasonic standing wave. Early works studied is the alignment of several types of microparticles and live cells in bulk metal channels [9-10]. Bulk metal channels are not so appropriate for lab-on-a-chip applications because of their difficulty to manufacture planner structure or wall surfaces inside a very narrow microchannel. On the other hand, Polydimethylsiloxane (PDMS) is a widely used polymer for microfluidic channels because of its favorable mechanical and optical properties [18] as well as its simple fabrication process. Also, metal surfaces have non-specific adsorption of reagent/sample molecules from the surrounding fluid (so called “biofouling”), which is not desirable for biological assays and dilute samples. PDMS is free from this befouling effect. That is why; nowadays much interest has been received in manipulation of microparticles in PDMS microfluidic channels. In this regard, several manipulation experiments were carried out recently [19-20] where Franke et al. [19] manipulate HaCaT cells (human keratinocytes) and Afroja et al. [20] successfully aligned the Cyanobacteria in a PDMS microfluidic channel using ultrasonic radiation force.

E.coli is the most widely studied prokaryotic model organism and it is an important species in the fields of biotechnology and microbiology. If we can manipulate E.coli bacteria using ultrasonic radiation force it will be a milestone for many biotechnological research field. Thus in this work, our concern is the ultrasonic manipulation i.e. ultrasonic alignment of E.coli bacteria in the PDMS microfluidic channel. E. coli bacteria which expressing green fluorescent protein (GFP) was used as the test sample. The successful alignment of E.coli bacteria in the nodal plane of ultrasonic standing wave along with the other previous study proved that ultrasonic radiation force has the wide range of manipulation capability of several types of live cells like Cyanobacteria, E.coli bacteria etc which can be used for further treatment in bioassays.

2. THEORETICAL BACKGROUND

If a particle suspended in a fluid in a microfluidic channel and a standing wave can be introduced in that channel, the particle will experience an ultrasonic radiation force derived by Yosioka et al. [21]. This radiation force will compelled the particle to move to the nodal or antinodal plane of the standing wave depending on the density and compressibility of particles and suspended medium. The ultrasonic radiation force equation derived by Yosioka et al. [21] is given below:

$$F_r = -\left(\frac{\pi P_0^2 V \beta_m}{2\lambda}\right) . \phi(\beta, \rho) . sin(2kx) \quad (1)$$

Where, P_0, V, λ, β, ρ, k, x are acoustic pressure amplitude, particle volume, wavelength of the ultrasonic wave, compressibility, density, wave number, and distance from the nodal plane respectively. $\phi(\beta, \rho)$ is called the contrast factor which is given by the following equation:

$$\phi(\beta, \rho) = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m} \quad (2)$$

Here the subscripts *p* and *m* denote the particle and liquid medium, respectively. If the contrast factor is positive, then the particles will move to the nodal plane, but if the contrast factor is negative, then the particles will move to the antinodal plane. Generally most rigid particles and cells suspended in water have positive contrast factors, however, some low density particles such as air bubbles; lipid particles etc. have negative contrast factors.

3. MATERIALS AND METHODS

3.1 Live cells (E.coli bacteria)

E.coli (Escherichia coli) is a gram-negative, facultative anaerobic, rod-shaped bacterium which is commonly found in the lower intestine of warm-blooded organisms. E.coli bacteria cells have the ability to survive outside of the body for a limited amount of time. This property makes them an ideal indicator organism to test environmental samples for fecal contamination [22]. It has been examined that environmentally persistent E. coli can survive for extended periods outside of the host [23]. That is the reason it is an important microorganism in the fields of bioscience and biotechnology. Also, the bacteria can be grown easily and inexpensively in a laboratory setting. It has been intensively investigated for over 60 years where it has served as the host organism for the majority of work with recombinant DNA. In this experiment, E. coli bacteria which expressing green fluorescent protein (GFP) was used as the test sample.

3.2 Generation of ultrasonic force by two transducers

A standing wave, in which particles in fluid were trapped at nodes and antinodes of the ultrasonic pressure field, can be generated between a transducer and a reflector [9]. In this case, the channel wall usually acts as a reflector. However, a channel wall made of soft materials such as PDMS cannot act as a perfect reflector due to its poor acoustic reflection properties. So, in this work, two transducers were used from opposite sides to generate ultrasonic waves inside a soft polymer like PDMS microfulidic channel. [20]

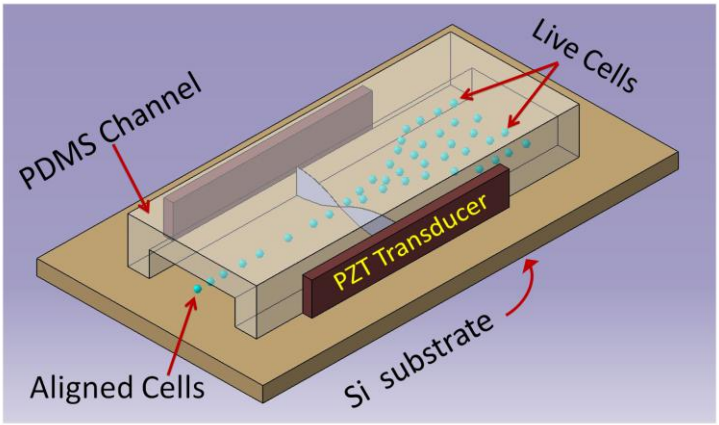

FIG 1. Schematic of microparticle alignment procedure in a PDMS microfluidic channel fabricated on a Si substrate.

3.3 Experimental setup

Figure 1 shows the schematics for the alignment procedure in a PDMS microfludic channel using ultrasonic standing wave. Here two commercially available PZT transducers (Panametrics NDT, A330S, 0.25″×1.5″) with 2.25 MHz center frequency were used to excite ultrasound inside the channel. The transducers were attached to both sides of the channel using liquid couplant (Ultragel II, Sonotech, USA) for better ultrasonic transmission. Each transducer was connected to a function generator (Agilent 33250A, Agilent Technologies, Inc.) through a power amplifier (AG1017L, RF Power Amplifier, T&C Power Conversion, Inc.). Only one function generator was used to synchronize the applied signals generated from both transducers. A syringe pump (Fusion 200, Chemyx Inc., USA) was used for injecting the E.coli bacteria into the fluidic channel using tubing attached to the inlet and outlet of the fluidic channel. A laminar flow of the suspension was used to inject the sample to the channel. The PDMS microfluidic channel was fabricated using soft lithography technique [20]. According to Figure 1, we see that the width of the channel should be equal to the half wavelength (λ/2) of the corresponding applied signal frequency to achieve the resonance condition inside the channel. Thus the width of the channel was chosen to 330 µm which is equal to the half wavelength (λ/2) of the corresponding center frequency of the transducer i.e. 2.25 MHz. Finally, the alignment was observed through an optical microscope (Eclipse LV150, Nikon Instruments Inc., USA) and fluorescence images were taken using a CCD camera (ARTCAM-300MI-DS, Artary Co. Ltd., JAPAN) which was connected to a personal computer.

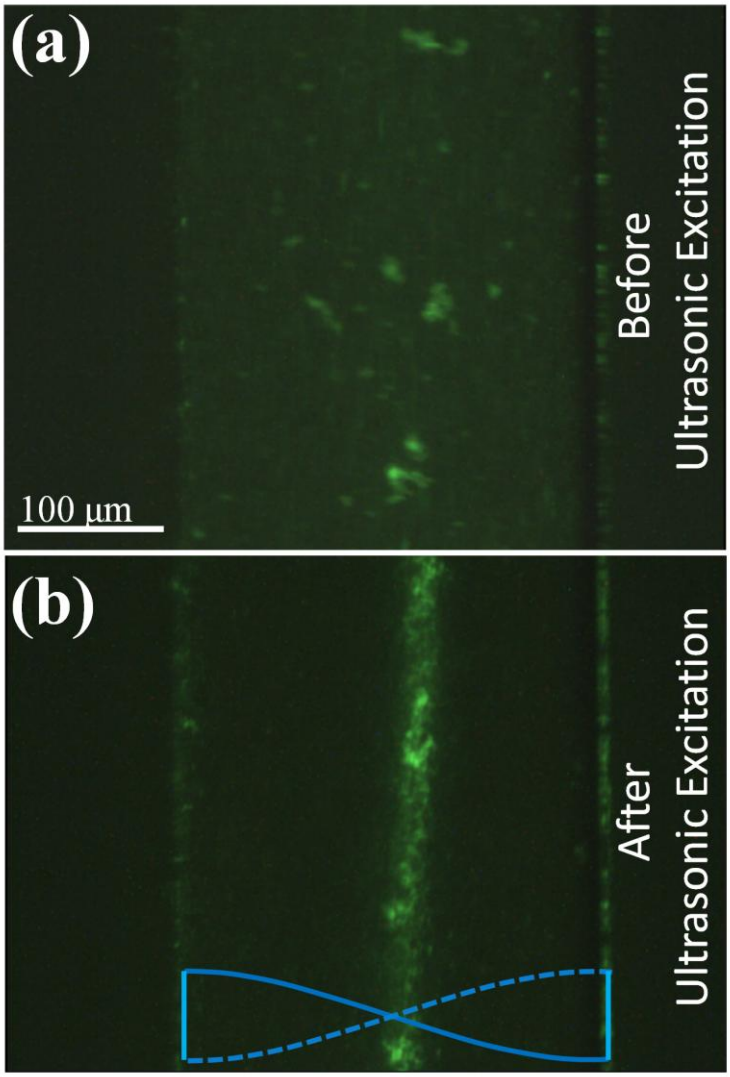

FIG 2. Alignment of E.coli bacteria in a PDMS microfluidic channel. (a) Random distribution of E.coli bacteria before ultrasonic excitation (b) Aligned E.coli bacteria in the nodal plane of ultrasonic standing wave after ultrasonic excitation.

4. RESULTS AND DISCUSSION

It has been already explained that E.coli bacteria plays an important role in the research field of biotechnology and microbiology, so we executed the manipulation experiment like alignment of E.coli bacterial in the nodal plane of ultrasonic standing wave. Figure 2(a) shows the distributed green fluorescent E.coli bacteria in the PDMS microfluidic channel before ultrasonic excitation. After ultrasonic excitation, the radiation force exerted on the E.coli bacteria towards the direction of nodal plane of ultrasonic standing wave. After a very short time, most of the E.coli bacteria get aligned in the nodal plane of ultrasonic standing wave inside the bio-compatible PDMS microfluidic channel that is shown in Figure 2(b). From the visual inspection it can be said that almost all cells can be sorted from the medium and concentrated in a single plane. However, some of the cells might stick to the channel wall or to the Si window, which act as a substrate here, due to the physical properties of cell and channel wall. Nonetheless, this efficient alignment result clearly reveals that the cells are highly concentrated in the plane which can prove that ultrasonic radiation force has the great capability in cell manipulation.

5. CONCLUSION

We have successfully aligned E.coli bacteria in a bio-compatible PDMS microfluidic channel attached on a Si substrate using ultrasonic radiation force. Two ultrasonic waves, approaching each other, were used to a generate standing wave inside the channel. This causes the radiation force inside the channel along the direction of nodal plane of ultrasonic standing wave and hence aligns the cells in the nodal plane. The effective and efficient alignment in a plane proved that ultrasound has the capability to manipulate various types of live cells like cyanobacteria, E.coli bacteria etc. So, this ultrasonic manipulation can be applied to a concentration of several types of live cells which is a pre-requisite for various lab-on-a-chip bioassays.

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