Micro-piezo Actuator for Cell Lysis

Sushama Agarwalla and Suhanya Duraiswamy(✉)

Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Hyderabad, India
suhanya@che.iith.ac.in

Abstract. In this study, we demonstrate a piezoelectric actuator for the lysis of bacteria using Travelling Surface Acoustic Waves (TSAWs) produced by gold IDTs (Interdigitated Transducers) fabricated on lithium niobate substrate. We are able to achieve ~99% lysis using IDTs of width 50 μm each, at 19.9 MHz. The developed piezoelectric actuator is a chemical free technology and can be used to lyse any type of bacteria as well as eukaryotic cells.

Keywords: Cell lysis · Piezoelectric actuators · TSAW

1 Introduction

Cell lysis is a process that involves destroying or breaking down the outer boundary of a cell to release its intercellular contents, such as DNA, RNA, and proteins. Lysis is an important tool in the field of molecular diagnostics of pathogens and in the development of various laboratory equipment such as point of care tests and protein purification kits. In addition, it is a critical step in several diagnostic and therapeutic efforts, since many disease biomarkers are typically found within the cell membrane [1]. The presence of a multi-layered cell wall in microorganisms requires advanced processes to lyse them and some of the currently used processes along with their disadvantages are as follows: (1) Thermal lysis which is not suitable for all organisms and can damage certain heat sensitive proteins and nucleic acids. (2) Electroporation, known for its versatility and low DNA requirements though cell damage and nonspecific transport of molecules are some of the disadvantages associated with it. (3) Mechanical lysis, the most preferred technique, which typically involves shear forces to physically damage the cell membrane. (4) Chemical lysis, the most effective strategy, though chemicals might interfere with downstream processing. Although several such techniques can lyse bacteria, a single method is not widely used for all types of bacteria like gram negative (*E. coli, Pseudomonas*) and gram positive (*Staphylococcus, Bacillus*) [2, 3].

In this study, we have developed a piezoelectric actuator for lysis using TSAWs (Travelling Surface Acoustic Waves) that are produced by gold IDTs (Interdigitated Transducers) on lithium niobate substrate. A piezoelectric actuator is a type of device that converts electrical energy into mechanical stress and the lithium niobate substrate is the piezoelectric material used in our study [4, 5]. We believe that this technique can be used for the lysis of not only bacteria but also other microorganisms and eukaryotic cells, without compromising the quality of the released cytoplasmic content.

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2 Methodology

2.1 Sample Preparation

We use *E. coli* (DH 5 alpha) as a model for our study. We prepared an overnight bacterial culture (broth) and measured its optical density using UV-Vis spectrophotometer (UV1780, Shimadzu, Japan) at 600 nm. An absorbance 1 at OD_{600} was considered to be 10^9 CFU/ml where CFUs are colony forming units, to calculate the concentration of the culture.

2.2 IDT Fabrication

Fabrication schematic is depicted in Fig. 1. The lithium niobate wafer (128° Y-X cut, University Wafer, Inc. South Boston, USA) was first washed with IPA (Isopropyl alcohol) and blow dried, followed by sputter (AJA International, Inc. USA) deposition of 20 nm titanium and 80 nm gold layers. Then positive photoresist (S1813, Shipley, USA) was spin coated at 3000 rpm and baked for 1 min at 120 °C. 405 nm laser source was used to pattern IDT structure on the substrate. After the patterning, the positive resist was removed using MF319 developer (Dow, USA) while the gold and titanium coating were etched off to get the final IDT structures.

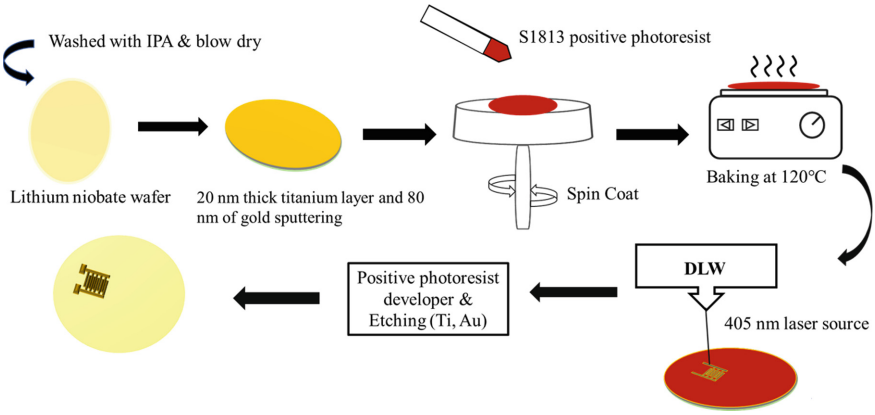

Fig. 1. Schematic of process flow for IDT fabrication

2.3 PDMS Microchannel Fabrication

The microchannel was designed in Auto CAD and printed using SLA (Stereolithography) 3D printer (Project 6000 HD, USA). The 3D printed wafer was then rinsed with IPA and further cured in UV chamber for 30 min to get the master patterns. Poly (dimethyl siloxane) (PDMS) (Sylgard 185, Dow, USA) replica was then moulded from these master patterns. The procedure in brief: PDMS base and cross-linker were mixed in 10:1 ratio

and degassed for 15 to 20 min. They were then poured onto the master patterns and cured in a hot air oven at 70 °C for 3 h. The PDMS layer was then peeled off from the master patterns, inlet and outlets holes punched and bonded to the lithium niobate substrate (having the fabricated IDT patterns) using a plasma cleaner (PDC-VCG-2, Harrick Plasma, USA) as shown in the schematic in Fig. 2.

2.4 Device Setup and Operation

The individual fingers of the IDTs were interconnected using bus bars as shown in the inset in Fig. 2. We used RF signal generator (DSG815, Rigol, China) in modulation mode to send pulsed, high-frequency signal to the IDT. Samples of required concentration (10^9 CFU/ml) were pumped through the PDMS channels using at various flowrates (from 1 to 20 μl/min) using a syringe pumps (Fusion 101, Chemyx Inc., USA). They were then collected and plated on LB agar (M1151, HiMedia, India) to determine the cell viability by colony counting (using a pen and a click-counter).

3 Results and Discussion

We fabricated a hybrid device with IDTs patterned to a piezoelectric substrate and a PDMS microchannel bonded onto the same substrate as shown in Fig. 2. The IDTs were fabricated onto a 128° Y-X cut lithium niobate substrate, which were excited to generate the surface acoustic waves. Bacteria were exposed to TSAW that can cause them to lyse, while they pass through the channel. The IDT patterns fabricated on Lithium Niobate substrate have parallel finger pairs of different finger width and gap and can operate at different frequencies. We calculated the SAW wavelength generated from different structures of IDT using Eq. 1;

$$\lambda_s = 2(w_i + g_i) \tag{1}$$

where, λ_s is SAW wavelength and *wi* and *gi* are the width of the finger and gap between the adjacent fingers of IDT respectively. We fabricated IDTs of 50 μm finger spacing and varied the frequency of the signal to the IDTs. The device can produce SAW wavelength of 200 μm (using Eq. 1). The operating frequency can be calculated by using Eq. 2

$$\mathrm{f_O} = v_s/\lambda_s \tag{2}$$

where, *fo* is operating frequency and v_s is the acoustic velocity of the substrate (3980 m/s for the current substrate).

The device can be used for an applied operating frequency of 19.9 MHz calculated using Eq. 2 for SAW wavelength of 200 μm [6, 7]. We varied the flowrate of the sample through the microchannel at constant applied frequency to analyze the effect on lysis. The experimental results are summarized in Fig. 3.

We also performed a control with 'TSAW off' at all flowrates and analyzed the cell viability. We see that majority of the cells are dead (cell lysis > 97%) after being subjected to TSAW, (indicated by black bars in Fig. 3a) for all flow rates tested. Cell lysis is ~100% at flowrates <5 μl/min (Fig. 3a) since the bacteria are exposed to TSAW

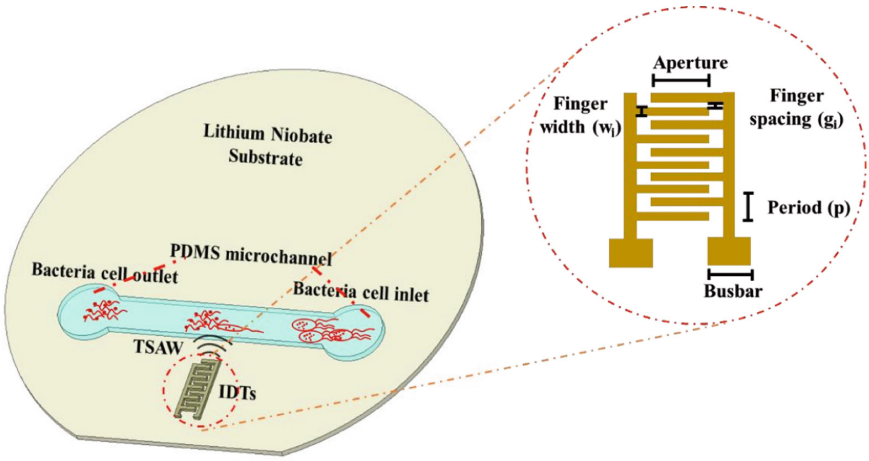

Fig. 2. Schematic of piezoelectric actuator for microbe lysis

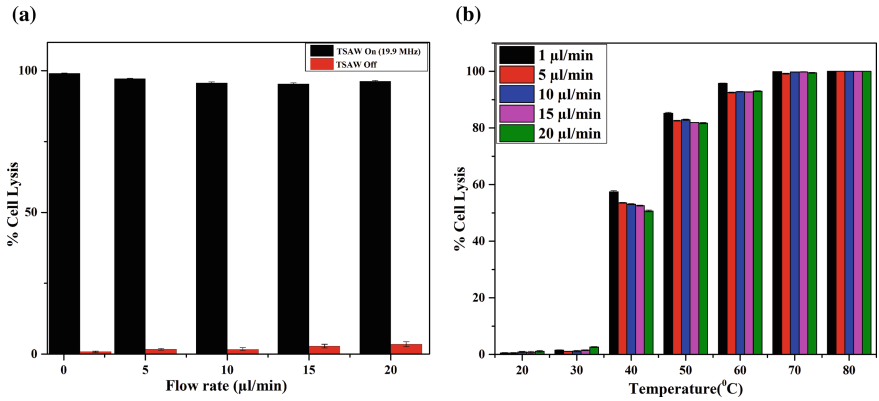

Fig. 3. Bar chart showing the lysis of bacteria (in %) plotted against (**a**) different flowrate at two different conditions TSAW on (black) and off (red) at 19.9 MHz frequency to generate 200 µm wavelength of TSAW (**b**) different temperature, without TSAW.

much longer (~13.7 s) at these flowrate. As the flowrate increases, to 20 µl/min, we observe a slight reduction in viability from ~0 to ~4% due to reduction in residence time (3.4 s).

We also observed temperature increase with TSAW application and performed an experiment to check the heating effect on lysis in the microchannel, as shown in Fig. 3b. We placed the hybrid device on a hotplate, pumped the bacteria through the microchannel, at varying flow rates, with 'TSAW off' at all conditions, and observed the cell lysis pattern. Cell lysis is low at 20 to 30 °C (~1–4%) while it increases from ~4% to 100% with increase in temperature from 30 °C to 80 °C. As observed previously, cell lysis was higher at lower flowrate and higher temperature (for example, ~53.5% at 5 µl/min and 40 °C as compared to ~50.6% at 20 µl/min and 40 °C). This again is due to the

increased residence time of bacteria in the microchannel thus, their increased exposure time to high temperature, affecting their lysis.

Experiments are in progress to determine the mechanism of cell lysis. The possible reasons for lysis of cells treated with SAW is the energy transfer to the cells that are located at the pressure nodes of the SAW waves as well as the temperature. Several factors such as the frequency, the power, and the volume of the cell suspension, flowrate, distance between the channel and the IDTs, will affect the efficiency of the lysis process. The other major challenge with the cell lysis component of the device is determining the right dimensions for the IDTs. Although the exact mechanism of the interaction between the device and the bacteria is not known, it is believed that the resulting cell lysis can be performed efficiently. We are working on the numerical simulation and will be fabricating IDTs based on the simulation result and will come up with the strategies of lysis of bacteria with varying IDT designs and input operating frequencies. The current study is a proof of principle for this particular work. In conclusion, this work shows in principle that diverse bacterial population can be lysed using SAW generated by IDTs fabricated onto a piezoelectric substrate and this can be a generic lysis strategy.

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