CELL LYSIS BY LOW POWER FOCUSED ACOUSTIC TRANSDUCER AND INVESTIGATION OF ACOUSTIC INTENSITY THRESHOLD FOR CYTOLYSIS OF VARIOUS CELL LINES

Lingtao Wang\textsuperscript{1}, Yi-Jia Li\textsuperscript{1}, Anderson Lin\textsuperscript{1}, Shih-Jui Chen\textsuperscript{1}, Mitchell Gross\textsuperscript{2}, and Eun Sok Kim\textsuperscript{1}

\textsuperscript{1}Department of EE–Electrophysics, University of Southern California, Los Angeles, CA, USA and \textsuperscript{2}Department of Medicine, University of Southern California, Los Angeles, CA, USA

ABSTRACT

This paper reports recent development of micron-sized cell lysis without noticeable acoustic cavitation by a Self Focused Acoustic Transducer (SFAT). Localized cell lysis was obtained with prostate cancer cell lines and breast cancer cell lines by applying various power intensity levels at the transducer’s fundamental and 3rd harmonic resonant frequencies (17.3 and 56 MHz, respectively). The cell lysis area was around 100 \(\mu\)m (fundamental) and 50 \(\mu\)m (third harmonic) in diameter. According to the results, the cell lysis area depended on the acoustic-wave frequency, but not on the applied acoustic intensity above the threshold intensity. Also, different cell lines showed varied sensitivity to focused acoustic energy, and had different energy threshold for localized cytolysis.

KEYWORDS: Self Focused Acoustic Transducer (SFAT), Localized Cytolysis, Cancer, Micro-Electro-Mechanical-System (MEMS)

INTRODUCTION

Cell lysis involves the process of rupturing the cell membrane, allowing intracellular components out of the cell and ultimately leading to cell death. Localized cell lysis with micron precision will provide unprecedented opportunities in cancer diagnosis and therapy at cellular level. Microfluidic systems for cell lysis that have been developed use thermoelectric lysis, electrical lysis, mechanical lysis, chemical lysis and ultrasonic lysis [1]. But these cell lysis platforms do not have location selectivity.

Conventional ultrasonic lysis or sonoporation uses strong ultrasonic waves, and relies on cavitating bubbles as focusing agents to concentrate energy and stresses [2]. These approaches are hard to control, because cavitating bubbles will undergo a fast collapse and generate heat and stress. In our previous study with an SFAT [3], we applied high power to generate the cavitation bubbles at the focal spot.

With low power intensity at the focal spot, violent cavitating bubbles can be avoided [4]. In this study, we designed and fabricated SFATs to work at its fundamental and 3\textsuperscript{rd} harmonic resonant frequencies. Then, we tested the minimum power requirement for cell lysis and corresponding acoustic power intensity within the focal spot. Three prostate cell lines (22RV1, RWPE-1, RWPE-2) and two breast cell lines (MCF-7, MCF-10) were treated with the focused ultrasound from SFATs with various acoustic intensities, in order to characterize cell’s sensitivity to ultrasound and acoustic intensity threshold for causing localized cytolysis.

DESIGN AND FABRICATION

The SFAT-based cell lysis device consists of a Lead Zirconate Titanate (PZT) transducer with an air-reflector acoustic lens, an acoustic chamber and a cell culture chamber (Fig. 1). Since the thickness of the PZT is 127 \(\mu\)m, the PZT’s fundamental resonant frequency \(f_0\) for the thickness mode is 17.3 MHz, while its 3\textsuperscript{rd} harmonic frequency is 56 MHz. Parylene lens with air reflectors on the PZT transducer focuses acoustic waves at the focal spot [5]. The height of the acoustic chamber is 800 \(\mu\)m, which is equal to the focal length of the acoustic lens. The acoustic chamber is filled with ultrasonic gel that has a good acoustic impedance matching with the PZT transducer. Monolayer prostate cell line is cultured on the membrane of parylene D in the cell culture chamber. The transparent parylene and silicon nitride membranes allow easy optical observation of cell condition with a fluorescent microscope.

For cell lysis, the PZT transducer generates acoustic wave in response to applied electric power. The Fresnel acoustic lens focuses acoustic waves by making the waves arrive at the target focal spot in phase. Thus, at the focus spot, the concentrated acoustic radiation pressure exerts great shear stresses on the cells and ruptures the cell membranes.

The cell lysis device is fabricated with the steps illustrated in Fig. 2 which shows brief processing steps to fabricate a PZT transducer with an acoustic lens and Si acoustic chamber, as well as the Si cell culture chamber.

The PZT transducer part is made of a 127-\(\mu\)m-thick PZT sheet (PSI-5A4E, Piezo Systems) with patterned circular nickel electrodes on both sides. The acoustic lens structure is fabricated through the following steps in sequence: 1) Spin and pattern 3-\(\mu\)m-thick sacrificial photoresist on the PZT substrate; 2) Deposit 4.5-\(\mu\)m-thick parylene D for the lens material, and etch release holes with RIE (Reactive Ion Etching) oxygen plasma; 3) Soak the device in acetone to remove the sacrificial layer, then, take it out and wait until the air gaps are completely dry; 4) Deposit an additional 3.5-\(\mu\)m-thick parylene D to cover the release holes. To fabricate the 800-\(\mu\)m-thick acoustic chamber, we use KOH wet etching with two 400-\(\mu\)m-thick silicon wa-
fers, and bond them to the PZT sheet.

Cell culture chambers are fabricated in a 400-µm-thick silicon wafer with 1-µm-thick Si<sub>3</sub>N<sub>4</sub> on both sides through the following steps in sequence: 1) Pattern the Si<sub>3</sub>N<sub>4</sub> on one side, and form the chamber structure with Si<sub>3</sub>N<sub>4</sub> diaphragm using KOH wet etching; 2) Dice the wafer into small chambers, and deposit 3 µm thick parylene layer all over the chamber chips to form the parylene/Si<sub>3</sub>N<sub>4</sub>/parylene membrane structure. In order to make the membrane in the chamber hydrophilic for good cell adhesion, we subject the parylene surface inside RIE oxygen for 10 seconds. The dimension of the cell culture chamber is 6×14×0.4 mm<sup>3</sup>.

To prepare the cell samples in the cell culture chamber, the chamber is sterilized with ultraviolet (UV) light for 30 minutes for good seeding of the cells. After the sterilization, a monolayer of prostate cancer cell line 22RV1 is grown on the parylene surface of the cell culture chamber, in a CO<sub>2</sub> incubator at 37 ºC. The cell density reaches 80% to 90% confluence within one day.

**TESTING SETUP AND EXPERIMENTAL PROCEDURE**

With the cell culture chamber stacked on the acoustic chamber, the monolayer cells are on the focal plane of the acoustic waves. To conduct the cell lysis experiment, a pulsed sinusoidal signal is applied to the transducer to produce pulses of acoustic waves. We use a pulse generator to trigger a sinusoidal function generator to create a pulsed sinusoidal signal with 0.050 - 5 V<sub>peak-to-peak</sub> and a pulse width of 1 s with a typical duty cycle of 50%. The frequency of the sinusoidal signal is varied from the fundamental resonant frequency of the transducer (17.3 MHz) to the 3<sup>rd</sup> harmonic frequency (56 MHz). The pulsed driving signal is amplified through an RF power amplifier, before being applied to the device, with the amplified voltage ranging from 1 to 30 V, which determines the power applied to the transducer. The cell lysis process is optically observed with a microscope.

To monitor the cell lysis, a cell-impermeant fluorescent nucleic acid dye SYTOX Dead Cell Stain (Invitrogen Inc.) is used. SYTOX nucleic acid stain is a high-affinity nucleic acid stain that penetrates cells with compromised membranes, but does not cross the membranes of live cells. If the cell membranes are compromised, the SYTOX stain can be visualized as nuclear staining under a fluorescent microscope. An additional fluorophore, CellMask (Invitrogen), is also used to visualize cell membranes in some experiments.

**RESULTS AND DISCUSSION**

Fig. 3b shows the lysed cells of 22RV1 cell line within the focal area, as well as the intact un-lysed cells outside of the focal area, when 0.150 W/cm<sup>2</sup> acoustic intensity was applied at the focal spot. The typical lysed area was around 100 µm in diameter, which did not increase proportionally with the applied acoustic intensity, as can be seen in Fig. 3c and 3d. Fig. 4 compares the sizes of the cell lysis areas produced by the devices working at the fundamental (Fig. 4a) and third harmonic (Fig. 4b) frequencies. In the case of the third harmonic devices, the acoustic intensity at the focal spot was 7.156 W/cm<sup>2</sup>, much higher than the intensity by the fundamental device. However, the focal spot of the 3<sup>rd</sup> harmonic device was around 50 µm in diameter, less than a half of the focal spot of the fundamental device.

Three prostate cell lines (22RV1, RWPE-1, RWPE-2) and two breast cell lines (MCF-7, MCF-10) were treated with the focused ultrasound from SFATs with various acoustic intensities, in order to characterize cell’s sensitivity to ultrasound and acoustic intensity threshold to cause localized cytolysis. As can be seen in the results summarized in Table 1, the prostate cancer cell line 22RV1 had the lowest threshold among the 3 prostate cell lines, and the threshold of the breast cancer cell line MCF-7 was much lower than that of MCF-10.
Table 1. Acoustic Intensity Thresholds for Cell Cytolysis of Various Cell Lines

<table>
<thead>
<tr>
<th>Acoustic Intensity Threshold (W/cm²)</th>
<th>Prostate Cell Lines</th>
<th>Breast Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>22RV1</td>
<td>0.15</td>
<td>MCF-7</td>
</tr>
<tr>
<td>RWPE1</td>
<td>0.60</td>
<td>MCF-10</td>
</tr>
<tr>
<td>RWPE2</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>MCF-10</td>
<td>2.21</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

In summary, focused-acoustic-wave cytolysis devices were fabricated and tested for localized cell lysis by varying the power levels, the acoustic frequencies and the cell lines. With SFATs operating at their fundamental and 3rd harmonic resonant frequencies, localized cell lysis was obtained on prostate cell lines and breast cell lines at various acoustic intensity levels. According to the results, the prostate cancer cell line 22RV1 had the lowest threshold among the 3 prostate cell lines, and the threshold of the breast cancer cell line MCF-7 was much lower than that of MCF-10. Thus, as we carried out cell lysis on a micron-sized focal spot with micron precision, we observed that prostate cancer cell line 22RV1 and breast cancer cell line MCF-7 were lysed easily by the focused ultrasound from a SFAT. These results demonstrate that the SFAT cytolysis devices offer unique opportunities for cancer diagnostics and therapeutics.

ACKNOWLEDGEMENTS

This material is based upon work supported by Robert and May Wright Foundation Research Award.

REFERENCES


CONTACT

*Lingtao Wang; email: lingtao@usc.edu*