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Temporal and Steady State Acoustic Field in a Cell Culture Well : Simulation

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***Abstract*— The present study was to understand the true power irradiated to the cell line cultured on a culture well, in relation to the nominal power from ultrasonic transducer, and to characterize the temporal variations of the acoustic pressure exerted on the cell. Numerical simulation was carried out for a ~~typical~~ culture well exposed to 1 MHz continuous ultrasound generated by a circular transducer contact underneath the well. The results showed that the ultrasonic pressure exposed to the cell layer in the well was 6.7 times larger than the nominal pressure of the ultrasonic transducer. The ultrasonic pressure in the transient period rose rapidly and was widely variable, and the temporal peak was even greater than that of the steady state period. This suggests that the cells undergo characteristically different ultrasonic exposure between the transient and the steady state period.**

I. Introduction

A culture well is employed for an in vitro study. When it is used for looking at biological responses of cells to ultrasound, care should be taken to understand the ultrasonic field developed in the well which is complex in general [1] [2] [4] [5]. The present study was motivated to answer the following questions: how is the nominal transducer power different from the true power irradiated to the cell line cultured on the well? how uniform is the ultrasonic pressure along with the cell line? and why are the cell responses more significant than the ultrasound with a certain duty cycle? In order to answer the questions, numerical simulations were performed using a finite element method for predicting an acoustic field in a culture well. The objectives of the present study were to understand the true pressure irradiated to the cell that is cultured on the well, in relation to the nominal pressure of ultrasonic transducer, and to characterize the temporal variations of the acoustic pressure exerted on the cell line.

II. Materials and Methods

In the simulation, a culture well was exposed to continuous ultrasonic wave of 1 MHz generated by the circular transducer contact underneath the well. The well was made of polystyrene. As displayed in Fig 1, it had the outer diameter of 11.77 mm which was the same diameter of the transducer. The height of the well was 3.39 mm, and the thickness of its bottom layer was 1.39 mm. The cell culture medium was acoustically regarded the same as water, and its height was fixed to the wavelength (λ=1.5 mm). The acoustic property of the polystyrene and the culture medium were obtained from Duck [3]. The simulation was carried out in time domain using PZFlex (ver 3.0, Weidlinger Associates Inc., USA) for the low intensity of 0.5, 1, 2 and 3 W/cm^2, often used in vitro study [6]. The mesh size was set to λ/32. The calculation continued for a relatively long time up to ~400 μs until the predicted field reached a steady state condition.

Figure 1. Geometry and size of the culture well considered in the present study. Note that water was taken as the culture medium in the simulation.

III. Results and Discussion

The typical acoustic field that has been predicted is shown in Fig 2. The pressure reaches its peak at the center on the bottom of the well, followed by very rapid decrease in the radial direction. The pressure variations along the central axis are plotted in Fig 3 for the 4 different nominal intensities of 0.5, 1, 2 and 3 W/cm^2. The ultrasonic pressure exposed to the cell layer on the bottom of the well was ~ 6.7 times (higher) of the nominal pressure of the ultrasonic transducer (see Fig 4).

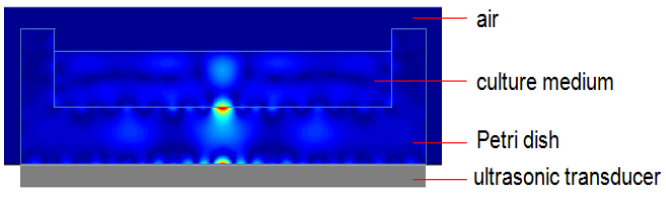

Figure 2. Cross sectional 2-D geometry of the cell culture well dish whose outer bottom was contact to the ultrasonic transducer.

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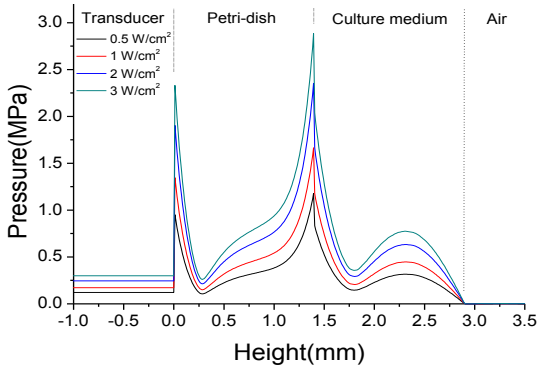

Figure 3. Pressure profile along the central axis of the culture well for the 4 nominal intensities of 0.5W/cm^2, 1W/cm^2, (c) 2W/cm^2, and 3W/cm^2. Note that the pressure maximum in the culture medium occurs at the height of 2.31 mm from the outer bottom of the well (located at 0.91 mm above the inner bottom of the well).

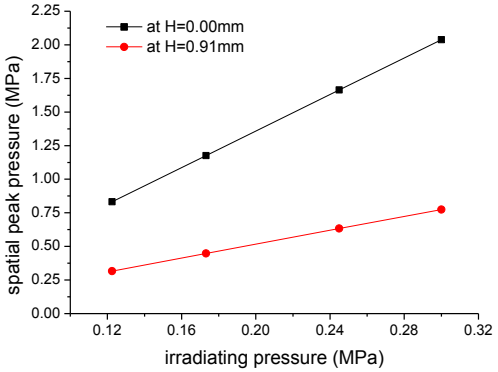

Figure 4. Spatial peak pressure predicted at the inner bottom of the culture well (H=0) and at the height where a local pressure maximum occurs (H=0.91mm), in relation to the nominal irradiating pressure at the surface of the ultrasonic transducer.

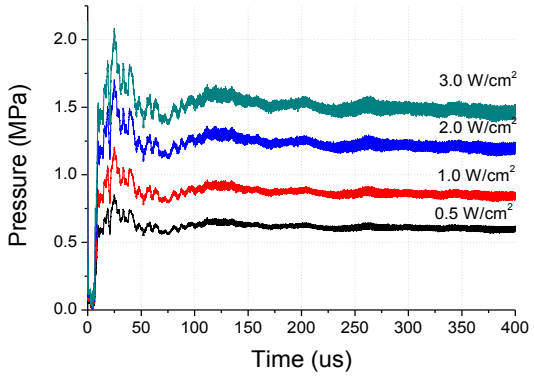

Figure 5. The envelops of the pressure waveforms predicted for 400 μs at the inner bottom of the culture well for the irradiating nominal intensity of 0.5, 1.0, 2.0 and 3W/cm^2, which were obtained using Hilbert transform. Note that the transient period lasts for about 150 μs, while the steady-state is apparent after 300 μs from the beginning.

Fig 5 shows the temporal variations of the magnitude of the pressures observed at the cell layer for the 4 different nominal intensities. It took several hundred times of the period of the wave until the acoustic field in the well reached steady state condition. The ultrasonic pressure in the transient period rapidly rose and was widely variable, and the temporal peak pressure was even greater than that of the steady state period. This suggests that the cells undergo characteristically different ultrasonic exposure between the transient and the steady state period.

Provided that the transient exposure is more effective way to stimulate cells, an optimum duty cycle would be closely associated with the duration of the transient period. Further studies are required to validate this issue. Nevertheless, our findings will be of use in a setting exposure condition when studying ultrasonic interactions with the cell lines cultured in a well.

IV. Conclusions

The acoustic field in the culture medium predicted in the present study showed that the exposure of cell layer in the well to the ultrasonic pressure was much larger than the nominal pressure of the ultrasonic transducer. Once ultrasonic irradiation was on, the temporal peak of the transient period was even greater than that of the steady state period. These findings will be useful in setting up a proper ultrasonic exposure to cells and may provide clues in understanding wide variability and poor repeatability in previous works with the culture wells exposed to the same nominal ultrasonic power.

Acknowledgment

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