An acoustic perfusion bioreactor for cultivating three dimensional cancer cell clusters

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***Abstract*—An optical microscopy compatible, scalable, acoustic standing wave perfusion micro-bioreactor has been designed and tested. Adherent HCT-116 colon cancer cells and non-adherent T cells have been examined. Bands of cancer cells were successfully trapped within acoustic standing waves and cultivated over several hours. Thermal generation of the acoustic ceramic was identified as a significant problem that was addressed by including a cooling chamber in the design. A system for the surface attachment and detachment of cancer cells within the bioreactor using thermally sensitive hydrogels and acoustic radiation forces is also proposed. The results of this study show that microscopic acoustic radiation pressure fields induced cancer cell clustering were in qualitative agreement with computer simulations. A fluorescent live dead assay confirmed the viability of the cells following several hours of cultivation in a temperature, CO_2, and humidity controlled optical microscope stage incubation chamber.**

Keywords- acoustic field; acoustophoresis; acoustic streaming; mammalian cell retention; optical microscopy; acoustic standing wave

I. INTRODUCTION

The successful cultivation of three dimensional tumor constructs has a number of important biomedical applications [1]. Increasing evidence supports the fact that two dimensional sheets of cancer cells are not representative of real tumors. Traditional hanging drop plates and non-adherent surface cultivation wells are a challenge to perfuse making dynamic pharmacological testing difficult. Alternative methods for testing chemotherapeutic agents on three dimensional tumor constructs from patient derived cancer cells are needed.

The development of an acoustic based bioreactor systems has a number of addition advantages over traditional bioreactors. Acoustic dewatering and further processing can also be potentially included. In addition, acoustic standing waves are capable of cell retention and increasing the efficiency of viral transduction. The successful design of a perfused acoustic standing wave bioreactor, however, has many technical challenges. In particular, the ceramic generates heat that can damage cells and produce convection currents.

This study outlines the design of an acoustic bioreactor. To deal with the PZT-8 heat generation, a cooling chamber is incorporated into the design. The complete assembly is compatible with an inverted fluorescent microscopy system. Cell viability is demonstrated using cancer cells with a fluorescent live/dead assay. Thermally sensitive hydrogels and an acoustic radiation force detachment system is included for the controlled attachment and detachment of cancer cells.

II. METHODS AND MATERIALS

A. Acoustic Bioreactor

Figure 1 shows a three dimensional computer aided design model (Dassault Systems SolidWorks 2015) of the acoustic bioreactor. A rapid prototype (Objet Eden500V) rendered a three dimensional prototype of the design (Objet FulCure 720). A laser cutter (Trotek Speedy 100) cut fused silica wafers (University Wafer 100mm DSP 500μm U01-120920) into the appropriate sizes for attachment to the bioreactor using epoxy (Loctite AA 3311) which was cured with a UV light source. A 3 MHz PZT-8 (APC International) transducer was attached to one surface using epoxy. The positive leads to the PZT-8 ceramic were attached using silver epoxy (MG Chemicals) that was cured at 150°C for 10 minutes. An impedance analyzer (Sine Phase 1-16,777 kHz) measured the PZT-8 ceramic impedance between 2.5 and 3.5 MHz before and after attachment to the bioreactor. A function generator (Agilent 33210A) drove the bioreactor ceramic with a $10V_{p-p}$ 3.5MHz sinusoid. The bioreactor was perfused using a syringe pump (New Era Syringe Pump NE-1000).

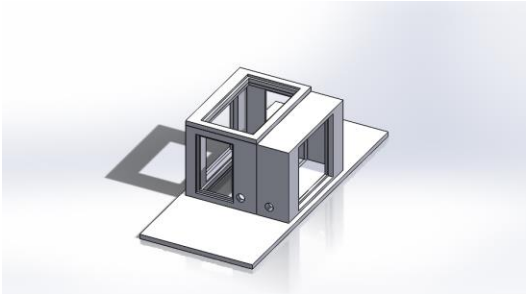

Fig. 1. Acoustic bioreactor. A PZT-8 transducer driven at 3 Mz produced standing acoustic waves within the bioreactor for trapping cells. A cooling chamber next to the transducer prevented heat generation from destabilizing the bioreactor.

B. *Cell culture*

Non-adherent Jurkat T cells (ATCC CRL-2899) were cultivated in T75 flasks (Corning 430641U) using RPMI 1640 (ATCC 30-2001), 10% fetal bovine serum (ATCC 30-2020), and 1% penicillin and streptomycin (ATCC 30-2300). Adherent colorectal cancer HCT-116 cells (ATCC) were cultivated in T75 flasks (Becton Dickenson) using McCoy's 5A with L-Glutamine (ATCC) and 10% fetal bovine serum (Hyclone), and penicillin and streptomycin (GibcoBRL). Cells were counted using a hemocytometer and initially screened for viability using 0.4% trypan blue stain (Gibco 15250-061). All cells were cultivated in an incubator (Thermo Scientific Hera Cell 150) at 37°C and 5% CO_2.

C. *Microscopy*

Live cell images were acquired using an inverted microscope (Zeiss, Axio Observer Z1) ccd camera (Hammamatsu, Orca-Flash 4) and incubation enclosure (Okolabs, Cage incubation system) with a temperature controller and CO_2 control unit (Boldline). A fluorescent live/dead assay (Molecular Probes L3224) was used to examine the cell viability.

III. Results And Discussion

Figure 2 shows the measured impedance spectra from the acoustic bioreactor filled to different levels. As the fluid level is increased, the overall shape of the impedance spectra remained the same. At specific fluid filled heights, however, additional resonance peaks were observed superimposed on the original impedance spectra.

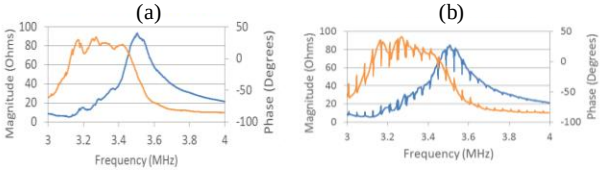

Fig. 2. Impedance spectra of acoustic bioreactor chamber half-filled and completely filled. (a) The impedance spectra with the chamber half-filled showed well defined anti-resonance peaks. (b) With the chamber completely filled a series of additional resonance and anti-resonance peaks were observed.

Figure 3 shows a simulation of the acoustic bioreactor. A two dimensional cross section of the acoustic bioreactor filled to different levels reveals two sets of resonances; one set in the horizontal direction and the other in the vertical direction. This was consistent with the observed cluster of cells in the acoustic bioreactor shown in Fig. 4. Clusters formed within minutes were stable after several hours of incubation. The green stained cells indicate intracellular esterase activity and the few red stained cells indicate cellular membrane breakdown.

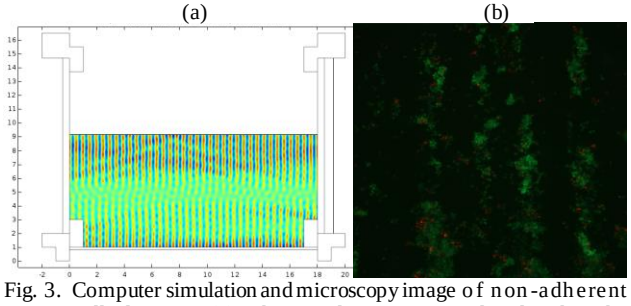

Fig. 3. Computer simulation and microscopy image of non-adherent cancer cell clusters. (a) The standing waves produced within the bioreactor were a function of both the bioreactor dimensions and fluid height. (b) Well defined cancer cell clusters are produced within the acoustic fields. Viable cells are stained green and dead cells orange.

Figure 4 shows the morphology of adherent HCT-116 cancer cells during a series of controlled detachment studies. A transducer placed on the bioreactor bottom produced acoustic radiation forces and cavitation effects to remove cells. Cells cultured on thermally sensitive N-isopropyl acrylamide based hydrogels are also being investigated to thermally control cell-surface adhesion.

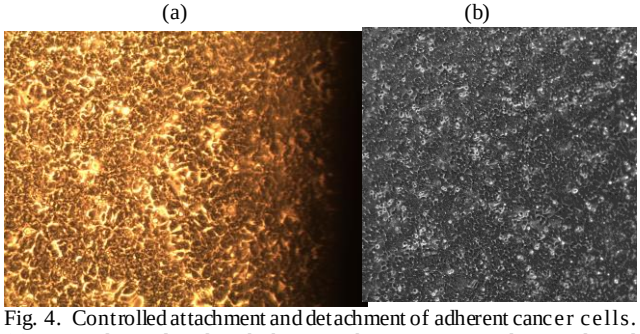

Fig. 4. Controlled attachment and detachment of adherent cancer cells. (a) A transducer placed on the bottom is being investigated to produced acoustic radiation forces and cavitation effects to remove attached cells. The shadow to left is produced by the transducer. (b) Cells cultured on thermally sensitive N-isopropyl acrylamide based hydrogels are also being investigated to thermally control cell-surface adhesion.

IV. Conclusion

An acoustic cancer cell bioreactor has been designed, implemented and tested using cancer cells. The results of this study demonstrated the formation of cancer cell clusters within and acoustic field. Cell viability was confirmed using a fluorescent live dead assay after six hours of cultivation.

Acknowledgment

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References

[1] G. Dane, K. Roy, R. A. Milton, S. Lichtner, R. Brindle, and L. Pack, Achieving large-scale, cost-effective, reproducible manufacturing of high-quality cells, A technology roadmap to 2025, National Cell Manufacturing Consortium, 2016.