Drug Sensitivity of 3D Cancer Cultures in Bioreactor with Micropillar Oxygen Delivery

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Introduction

Presently, we are using a novel approach to test drug sensitivity of 3D cancer cell culture growth *in vitro*. We utilize a bioreactor system that is designed to offer control of oxygenation to the 3D cancer cell cultures, in order to better mimic tumour microenvironments observed *in vivo*. Through the use of the bioreactor, it is hoped, we will better understand how cancerous tumour will react to chemotherapy drugs *in vivo* via this *in vitro* method

Traditional 2D (monolayer) cell culture is limited in its ability to provide an accurate perspective of the *in vivo* environment, not only because the standard atmospheric conditions used are 21% O_2, 5% CO_2, and ~74% N_2, which differs greatly from *in vivo* conditions, but also due to fundamental differences such as varying cell matrix composition. While cell culture is entirely possible using a variety of conditions that do not replicate *in vivo* conditions, such modifications will invariably lead to differing outcomes with regard to cell morphology, signaling, and gene expression, as has been widely observed [1-3].

In this experiment we analyzed the differences in performing cytotoxicity assays on cell cultures grown in both 2D and 3D conditions, looking for differences in sensitivity to chemotherapy drugs based on the respective IC-50s of cells grown in those conditions. Two cancer cells lines, OVCAR8 (breast) and MCF7 (ovarian), were examined with 3 different drug applications, Adriamycin, Cisplatin, and Taxol, in the 2D assay. Due to compressed time schedule only OVCAR8 under an Adriamycin drug condition was tested in the 3D condition to be compared.

Membrane Micropillar Structure

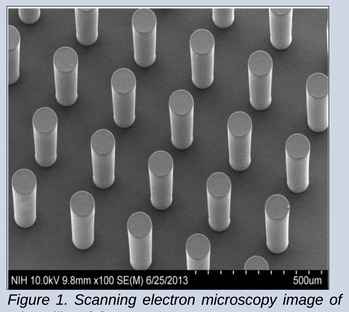

Figure 1. Scanning electron microscopy image of micropillars [5].

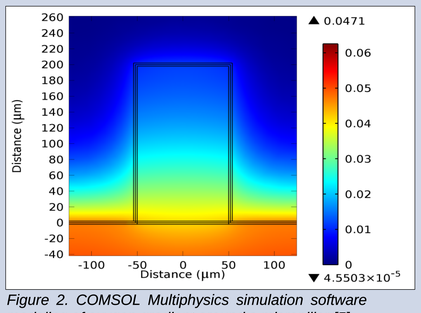

Figure 2. COMSOL Multiphysics simulation software modeling of oxygen gradient around a micropillar [5].

Bioreactor

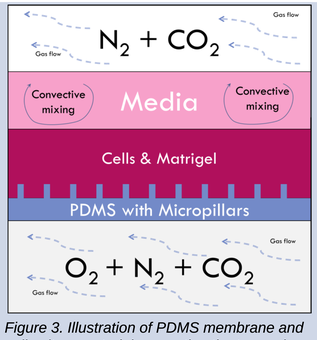

Figure 3. Illustration of PDMS membrane and cell culture material separating the top and bottom chambers.

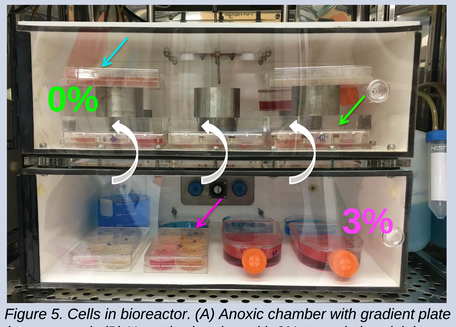

Figure 5. Cells in bioreactor. (A) Anoxic chamber with gradient plate (green arrow). (B) Hypoxic chamber with 3% control plate (pink arrow). (C) Anoxic chamber with 0% control plate (cyan arrow)

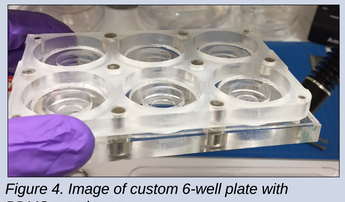

Figure 4. Image of custom 6-well plate with PDMS membrane.

4 Oxygenation conditions examined:
0% & Gradient O_2 (anoxic/top chamber),
3% O_2 (hypoxic/bottom chamber),
21% O_2 (incubator)

2D vs. 3D Culture Drug Sensitivity

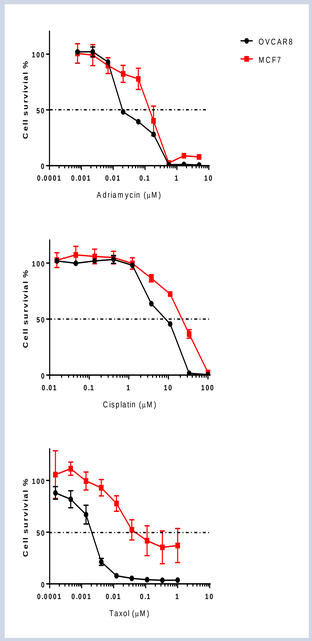

Figure 7. Cytotoxicity assay results of OVCAR8 and MCF7 cell lines in 3 different drugs: (top) Adriamycin, (middle) Cisplatin, (bottom) Taxol.

- 2D Cytotoxicity assay performed in 96-well plate for OVCAR8 and MCF7 cell lines
- 3 drugs used, each w/ different max concentrations: Adriamycin @ 5µM, Cisplatin @ 100µM, and Taxol @ 1µM
- Cell lines are plated, 5*10^3 cells/well, and let proliferate for one day, following day drug, is added. Cells let sit in drug+media solution for 3 days
- Cells counted using Cell Titer Glo
- Results represented as average percentages of 3 technical replicates per cell line compared to controls respectively +/- SD per drug
- OVCAR8 found more sensitive to all tested drugs than MCF7, so used in following 3D cytotoxicity assay

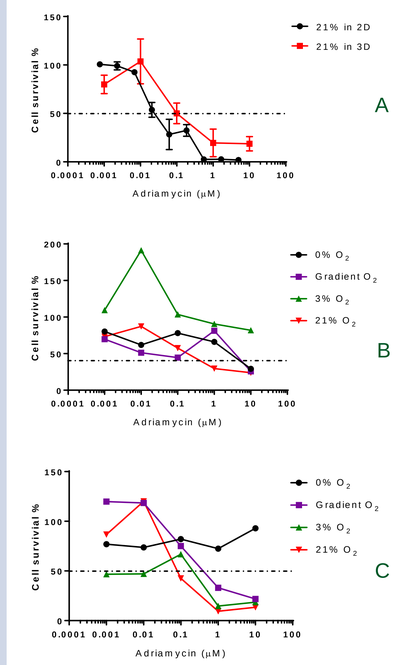

Figure 8. cytotoxicity assays on 3D cultures

- 3D cytotoxicity assay performed with Adriamycin in 6-well plates, initial cell count 6x10[4] cells per well
- Figure 8A: 21% O_2 condition: cells proliferate for 3 days, drug added on 4[th] day, cells counted on day 8. Results plotted as average of two technical replicates per condition compared to controls +/- SD.
- Figure 8.B and Figure 8.C: cells proliferate for 7 days, drug added on 8[th] day, cells counted on day 11. Figure 8B: 1mL media volume per well; Figure 8C: 5mL media volume. One measurement per condition.
- Cells counted using Cellometer Vision Trio 5
- Experimental problems led to transient loss of atmospheric control for several periods lasting a few hours; nonetheless, clear differences between oxygenation conditions are observed.
- 3D confocal image of cell growth in different drug concentrations in gradient O_2 condition
- Cell growth seen as a monolayer, that is up and branching between pillars in both conditions

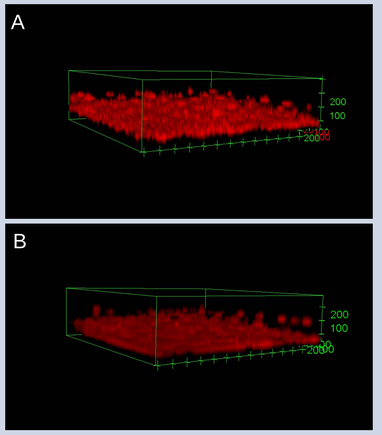

Figure 9. (A) Gradient O_2 sandwich w/ 10µM Adriamycin in 5mL drug+media (B) Gradient O_2 sandwich w/ .001µM Adriamycin in 5mL drug+media. Scales in µm

Cell Culture Distribution

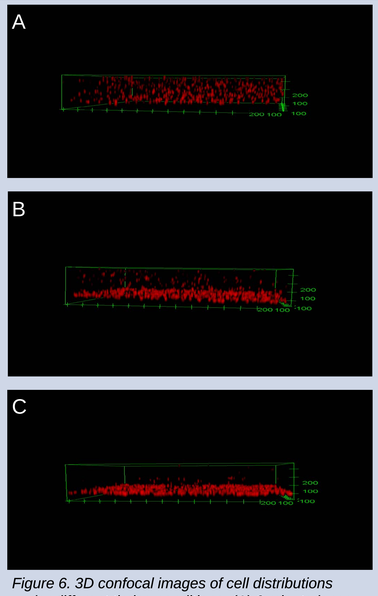

Figure 6. 3D confocal images of cell distributions under different timing conditions; (A) 0 minute lapse, (B) 10 minute lapse, (C) 20 minutes before insertion/flipping in 37°C. Scales in µm. Bottom of green bounding box indicate bottom of well.

- Examine differences in hold-time between when cell+Matrigel solution plated and when they were plate is put in the incubator for the flipping protocol.
- Flipping of the plates critical to preventing the cancer culture from growing as a 2D (monolayer) culture on the bottom of the PDMS membrane well
- Important to create a reproducible distribution of cells around the pillars in the Matrigel
- OVCAR8 cells, stably transfected with DsRed2 used for fluorescence under microscope
- 3 hold-time conditions used as prelude to the flipping procedure: 0, 10, and 20 minutes
- Imaging occurred immediately (1-2 hours) after cells were plated
- 0 minutes hold-time cells (*Figure 6.A*) suspended throughout the Matrigel
- 10 and 20 minute hold-times (*Figure 6.B & Figure 6.C*) suggests cells in monolayer on the floor of the well w/ some suspended throughout the Matrigel
- Observed past 10 minutes, a reproducible distribution of cells around the pillars is formed, so 10 minutes hold-time used in 3D cytotoxicity assay

Conclusions & Future Directions

- Performed first drug sensitivity experiments on 3D cell cultures using the bioreactor
- Results from initial cytotoxicity assays suggest differences in cell proliferation and drug response between differing oxygenation conditions
- Across all drug applications, the OVCAR8 cell line is found to be more sensitive to all four of the drugs than MCF7 as it is seen to reach IC-50 before that of MCF7 (*Figure 7*)
- Cell cultures grown in the 3D 21% O_2 condition, by this preliminary experiment, appeared less sensitive to Adriamycin than that grown in the 2D cytotoxicity assay (*Figure 8.A*)
- Developed protocol for reproducible cell distribution in Matrigel; further optimization could control cell localization within micropillars
- Further in-depth drug sensitivity studies with multiple technical and biological replicates over both OVCAR8 and MCF7 cell lines with multiple drugs.
- Refine cell collection and counting method for 3D cell culture

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