Optimization and Analysis of Recovered Cells from 3D Cancer Cultures with Micropillar Oxygen Delivery

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Introduction

Limitations of traditional two-dimensional (2D) monolayer cell culture [1-3]:
- Atmospheric conditions differ from physiological ones
- Mechanical and biochemical cues and cell-cell communication are lost
- Different cell morphology, signaling, and gene expression

Novel bioreactor system utilized:
- Oxygenation to 3D cancer cell cultures in order to better mimic tumor microenvironments observed *in vivo*
- Polydimethylsiloxane (PDMS) membrane with micropillars that deliver oxygen to the interior of the tumor spheroids and represent the structure and oxygen delivery within a capillary bed

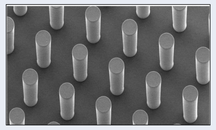


Figure 1. Scanning electron microscope image of micropillars approximately 200µm in height and approximately 75µm in width *[4].*

- Contains two atmosphere controlled chambers. Custom six-well plates sit between the two chambers and receive oxygen from the bottom through the PDMS membrane and from the top via diffusion through the media

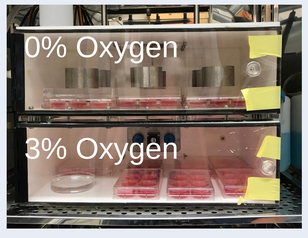


Figure 2. Image of the bioreactor system used in this study

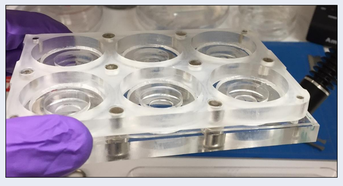
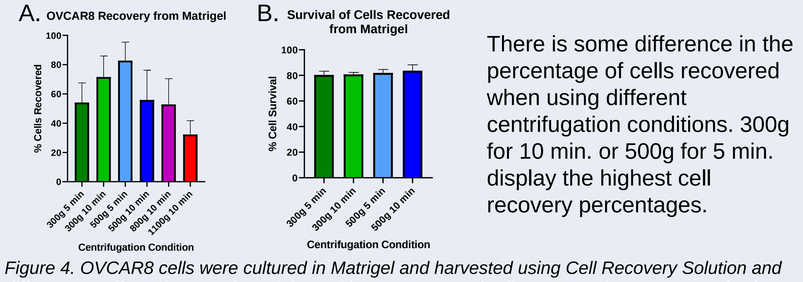


Figure 3. Image of the 6 well plate used in bioreactor with PDMS pillar membrane.

Challenges with harvesting cells:
- Traditional cell harvesting techniques aren't feasible because the cells are suspended in Matrigel and growing in spheroids
- Method to extract the cells alive was optimized in this study by using Cell Recovery Solution and different centrifugation conditions to spin the cells down into a pellet

After harvesting cells:
- Flow cytometry was used in order to find out more information about the cells, such as cell death and cell cycle due to different culture conditions and drug concentrations

Cell Recovery



There is some difference in the percentage of cells recovered when using different centrifugation conditions. 300g for 10 min. or 500g for 5 min. display the highest cell recovery percentages.

Figure 4. OVCAR8 cells were cultured in Matrigel and harvested using Cell Recovery Solution and different centrifugation speeds and times. The percentage of cells recovered was measured using (A) a cell counter and (B) flow cytometry with Annexin-V.

Metabolic Activity

OVCAR8 cells were cultured in 2D and 3D in various oxygen concentrations for 7 days, then the cells were incubated with 3 different drugs for 3 more days. Cell viability was assessed using Cell Titer Glo 2D and 3D which measures ATP concentration, corresponding to metabolic activity.

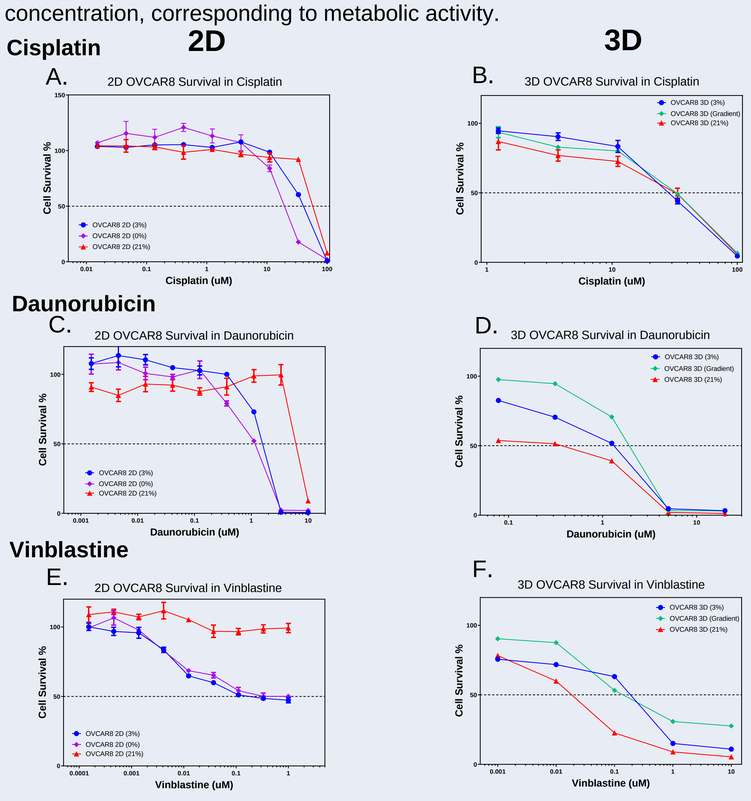


Figure 5. IC50 curves were constructed for (A,B) cisplatin, (C,D) daunorubicin, and (E,F) vinblastine.

- Daunorubicin and vinblastine affect the OVCAR8 cells differently depending on the oxygen concentration during culture.
- The drug efficacy for these drugs is different in 2D and 3D.
- In 3D, the cells grown in the oxygen gradient condition seem to be more resistant to the effects of the drugs than the other oxygenation conditions.
- The efficacy of cisplatin does not seem to be affected, or is only slightly affected, by the oxygenation condition of the cells.

Flow Cytometry

Flow cytometer measurements of recovered cells agree with *in situ* Cell Titer Glo measurements but can provide much more information.

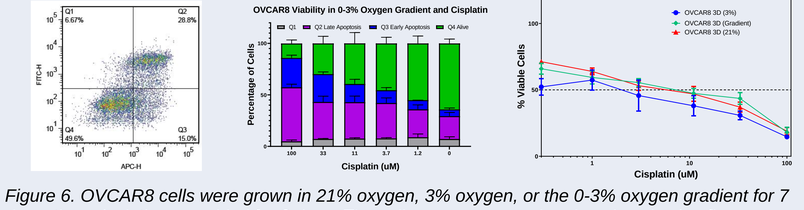


Figure 6. OVCAR8 cells were grown in 21% oxygen, 3% oxygen, or the 0-3% oxygen gradient for 7 days, then cisplatin was added for 3 days. The cells were recovered and analyzed using flow cytometry with Annexin-V and SYTOX Green. (A) Raw data scatter plot from the flow cytometry. (B) Stacked bar graph representing Annexin-V data from the gradient condition. (C) Graph of the percentage of viable cells measured using Annexin-V.

Cell Cycle Analysis

Propidium iodide staining of permeabilized cells is used to measure total DNA content in order to assess cell cycle profile.

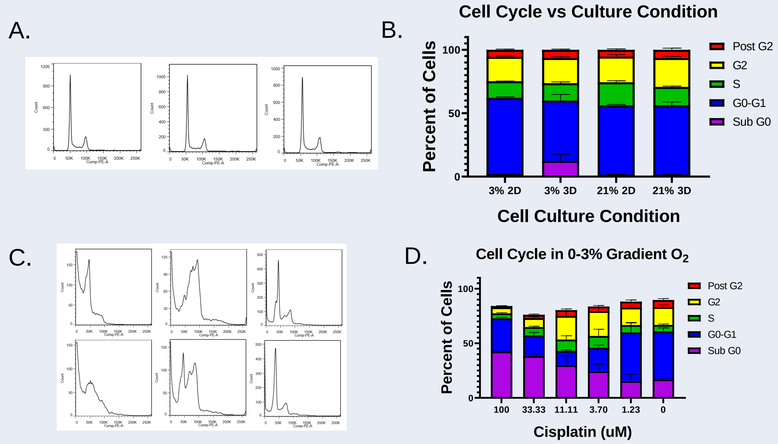


Figure 7. (A,C) Raw data and (B,D) stacked bar graphs of OVCAR8 cells analyzed using flow cytometry with propidium iodide to identify the cell cycle stages that the cells are in (A,B) when grown in a 2D monolayer and (C,D) when grown in 3D in various oxygenation conditions.

Conclusions

- The culture conditions of the OVCAR8 cells affect the efficacy of the drugs. There is an observable difference between the drug effects in 2D and 3D, and at the different oxygen concentrations.
- Cells can be successfully harvested and the flow cytometry data obtained is consistent with the *in situ* Cell Titer Glo, with some differences that can provide more information about the condition of the cells.
- Propidium iodide also has the potential of providing more information, but the data is obstructed by too much cell death using this protocol.

Future Directions

More information from flow cytometry:
- Multiplexing Annexin-V with propidium iodide and/or Ki-67

Spatial information:
- Histology: remove PDMS or section through it

Figure 8. Image of cells attached to PDMS pillar from immunohistochemistry attempt by Kris Yala and Stephen Hewitt in the National Cancer Institute

References

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Acknowledgements

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