3D Cancer Cell Culture with Controlled Oxygenation using Bioreactor and Microfabricated Pillars
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Introduction
Cells grown in a 2D monolayer do not mimic in vivo growth. A model of how cells grow in the body would enable better studies of complex diseases and treatment responses. The aim of this project is to grow cancer cell lines in 3D to create a model that more closely mimics in vivo behavior of tumors. There are three main components to being able to create such a model:
1. Controlled oxygenation
   - An oxygen gradient can be set up using a two-chambered bioreactor. The bottom chamber has 3% O₂ and the top chamber is anoxic (Figure 1.6). When cells are placed between these chambers, they experience a flow of oxygen from the hypoxic source to the anoxic one, much like cells would in vivo.
2. Synthetic vessels
   - The body’s vasculature is how cells receive O₂ and other nutrients. Current spheroid culture has reversed O₂ and molecular gradients than in vascularized tumors (Figure 2) [1,2]. Pillars therefore must be fabricated to act as the vessels for spheroids to grow around.
3. Cell-cell and cell-ECM interactions
   - Culturing the cells in Matrigel, an extracellular matrix (ECM), allows the cells to grow while interacting with other cells and an ECM, as actually happens in vivo.

Initial Cell Distribution Experiments
- Verify that current protocol optimizes 3D cell growth
- OVCAR8 line transfected with dsRed2 for fluorescence under microscope
- Imaging occurred immediately (1-2 hours) after cells plated
- Different plating conditions tested to see differences in settling (Figure 5)

Cell Growth Experiments
- OVCAR8-dsRed2 cells (6x10⁵ cells/mL) grown for 7 days in bioreactor (gradient O₂)
- Figure 6A, hypoxic chamber (3% O₂, Figure 6B), or in 37°C incubator (21% O₂).
- Spheroid density depends on oxygenation (Figure 7)
- Growth surrounding pillars observed (Figure 8)

Membrane Fabrication
- Cells embedded in 3mg/mL Matrigel and cultured onto PDMS membrane with micropillars on surface (Figure 3.4)
- Polydimethylsiloxane (PDMS) used for its high O₂ permeability
- Membrane fabricated via 3-step process involving photolithography (Figure 3) [1]
- Microstructured PDMS membrane clamped between magnetized acrylic pieces (Figure 4)

Cellular analysis
- Visual analysis using Partek Genomics Suite (Figure 9)
- TaqMan Low Density Array (TLDA) performed on extracted RNA to quantify differences in gene expression per condition

Membrane Fabrication
- Cells and Matrigel
- Media
- Proliferating zone
- Quiescent viable cell zone
- Necrotic core

Membrane Fabrication
- Cells and Matrigel
- Media
- Acrylic

Conclusions and Future Directions
- Cells successfully grown in Matrigel and bioreactor
- Preliminary experiments show differences in cell growth and gene expression based on oxygenation
- Further confirmation needed from more iterations and in-depth study of gene expression analysis
- Completion and analysis of OVCAR8-dsRed2 7 and 14-day experiment
- Drug penetration and multidrug resistance experiments
- Cell growth experiments with other cell lines
- Extracellular matrices other than Matrigel

References

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