Microfabricated polymeric vessel mimetics for 3-D cancer cell culture  
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Background & Introduction

The lack of vasculature in 3-D cell culture poses a significant barrier to engineering extended tissue constructs and mimicking in vivo molecular gradients.

3-D culture
• lacks vascularity and oxygen gradients
• discrepancies in gene expression, morphology, cell-cell and cell-matrix interactions, and differentiation compared to 3D culture

Animal models
• expensive to conduct trials
• may not sufficiently recapitulate features of human tumors, autoimmune disease, and drug response etc.

3-D cell culture (scaffolds and matrices)
• commonly mimic static, short-term conditions
• cell growth and migration into scaffolds limited by O2 and nutrient transport

Objectives

Aim 1: Engineer a microenvironment to “vascularize” 3-D cell culture by mimicking O2 perfusion from blood vessels via microfabricated pillar structures of photopolymerized silicone hydrogel (SilHy).

Aim 2: Evaluate effect of O2 gradients on growth patterns, gene expression, and pharmacologic response of tumor cells in the developed bioreactor system compared with conventional culture methods.

Engineering a Microenvironment

A bioreactor system was developed to facilitate gas exchange in cancer cell organotypic cultures using high aspect ratio micropillar structures. Given the central role of the microvasculature in supplying and limiting growth, we hypothesized that mimicking vessels using micropillars to facilitate delivery of O2 to surrounding cells would be more reflective of the in vivo tumor microenvironment.

Silicone Hydrogel Fabrication

Our approach utilizes a silicone hydrogel material with high O2 saturation and transmission properties. Silicone hydrogel was cast into a membrane with high aspect ratio micropillar structures, photopolymerized utilizing metathesis chemistry, and incorporated into the bioreactor culture system to deliver O2 through the hydrogel pillars into an otherwise hypoxic surrounding media.

Modeling the Microenvironment

A fiber-optic O2 sensor was used to experimentally model the dissolved O2 gradient in the bioreactor growth media as a function of distance away from a flat silicone hydrogel membrane (Fig. 8). To examine hypoaxia gradients within tumor spheroids cultured in the bioreactor, the hypoaxia marker Hypoxyprobe-1 (Pimonidazole HCl) was utilized for immunohistochemical analysis (Fig. 9).

Oxygen Gradient Analysis

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Bioreactor Tissue Culture

Fig. 10. Tumor hypoxia and cell proliferation status in 3-D cell culture at different O2 concentrations. OVCAR8-DsRed2 cells were cultured for 7 d at 6, 8, and 21% O2, in Matrigel and with 8% O2 in the lower chamber of the bioreactor culture system. Cell proliferation and hypoxia staining were performed with Ki-67 and Pimonidazole hydrochloride (Hypoxyprobe), respectively. Increasing O2 concentration showed a corresponding increase in Ki-67 staining and decrease in pimonidazole staining in 3-D Matrigel culture. The bioreactor culture demonstrates a difference in growth morphology and suggests that a lower O2 tension is required to achieve hypoxic culture conditions more akin to in vivo conditions.

Future Work

I sincerely thank my mentors, Thomas Pohida and Dr. Michael Gottesman for their extraordinary support and guidance. The culture system was designed and constructed at DEEKS. Microfabrication was performed at the Center for Nanoscale Technology at NIST and at the Microfabrication and Microfluidics Unit at NIBIB. Special thanks to Hynda Kleinman and Matthew Hoffman for their assistance with our use of Matrigel and the IRTA program for funding this research.