

# Microfabricated Polymeric Vessel Mimetics for Cancer Cell Culture

Ashley A Jaeger<sup>1,2</sup>, Chandan K Das<sup>2</sup>, Nicole Y Morgan<sup>3</sup>, Randy H Pursley<sup>1</sup>, Philip G McQueen<sup>1</sup>,  
Matthew D Hall<sup>2</sup>, Tom J Pohida<sup>1</sup> and Michael M Gottesman<sup>2</sup>  
Center for Information Technology<sup>1</sup>, National Cancer Institute<sup>2</sup>, National Institute of Biomedical Imaging  
and Bioengineering<sup>3</sup>, National Institutes of Health, Bethesda, Maryland, USA



## Background & Introduction

- Difficulties with current models for studying tumorigenesis and evaluating potential cancer therapeutics:

### 2D cell culture

- lacks vasculature and oxygen gradient
- considerable differences 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, stem cell differentiation, and drug response

### 3D cell culture (scaffolds and matrices)

- commonly mimic static, short-term conditions
- cell migration into scaffolds/membranes limited by O<sub>2</sub> and nutrient transport, leading to encapsulation

## Objectives

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

**Aim 2:** Evaluate effect of oxygen gradient on growth patterns, gene expression, and pharmacologic response of tumor cells in the developed bioreactor system and currently employed culture methods.

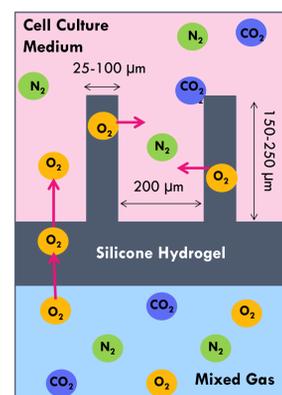


Figure 1. Model of oxygen diffusion through silicone hydrogel micropillars in the bioreactor environment.

## Engineering a Microenvironment

- A bioreactor system was engineered to mimic the physiological features of the *in vivo* environment, with multiple design considerations (Fig. 2). Environmental conditions, including gas flow (O<sub>2</sub>, N<sub>2</sub> and CO<sub>2</sub>), pH and dissolved oxygen level need to be precisely monitored and controlled.

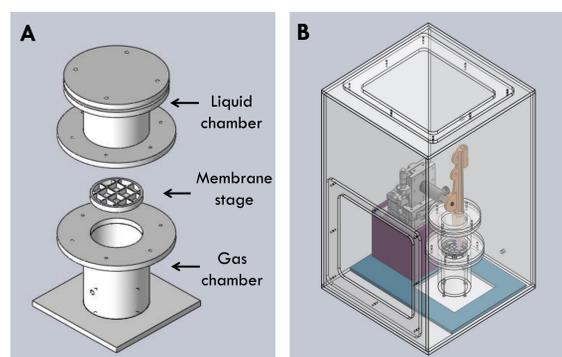


Figure 2. SolidWorks models of the 3D cell culture system. (A) novel bioreactor with mixed gas flowmeter inputs and (B) container to house the culture system with micrometer driven XYZ stage for precise measurements of measurements of oxygen and pH.

## Silicone Hydrogel Fabrication

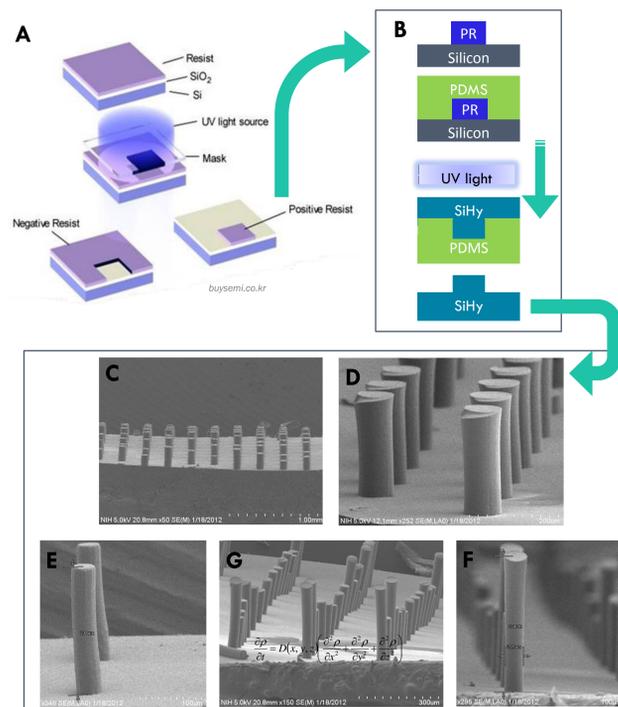


Figure 3. SiHy fabrication process. (A) Nanofabrication procedure used to create SU-8 photoresist (PR) mold for (B) double-casting process using polydimethylsiloxane (PDMS). (C-F) Hitachi S-4800 SEM images of SiHy membranes with (C-D) 100 μm, (E-G) 50 μm and (F) 25 μm diameter pillars up to 264 μm in height.

## Oxygen Gradient Modeling

Oxygen tension gradient values were measured with a microscale Clark-type electrode, allowing a 2D model of diffusion to be generated for O<sub>2</sub> tension ( $y$ ) as a function of distance from the hydrogel surface ( $x$ ) (Fig. 4). The resultant non-steady state model is:

$$y = 55.39e^{-1.185x} + 12.3$$

Matlab was used to create a steady state model of O<sub>2</sub> transport (Fig. 5) governed by the diffusion equation:

$$\frac{\partial \rho}{\partial t} = D(x, y, z) \left( \frac{\partial^2 \rho}{\partial x^2} + \frac{\partial^2 \rho}{\partial y^2} + \frac{\partial^2 \rho}{\partial z^2} \right)$$

$\rho$  = oxygen density  
 $D$  = Diffusion coefficient

With boundary conditions:

- bottom chamber held at fixed O<sub>2</sub> density
- top chamber is an oxygen sink

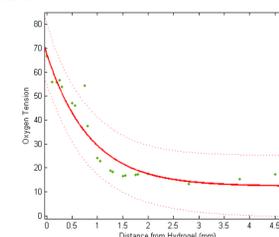


Figure 4. Oxygen tension gradient through SiHy membrane.

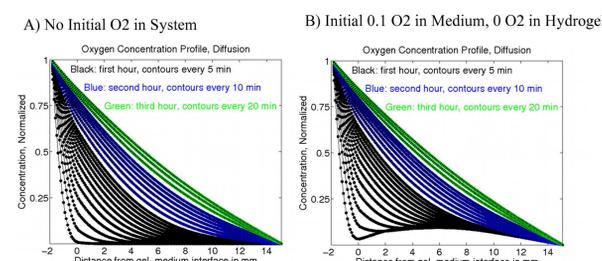


Figure 5. Matlab models of oxygen transport in the bioreactor system. The results indicate that it takes about 2-3 hours for O<sub>2</sub> levels to reach diffusion steady state in bioreactor.

## Synthetically Vascularized 3D Culture

- The bioreactor was seeded  $6 \times 10^5$  cells/mL with ovarian tumor epithelial cells in BD Matrigel or Trevigen Cultrex basement membrane extract (3 mg/ml). The bottom gas chamber was maintained at 85% N<sub>2</sub>, 5% CO<sub>2</sub> and 10% O<sub>2</sub> (76 mm Hg, compared to arterial levels of 38-114 mm Hg O<sub>2</sub>). The top chamber was maintained at 95% N<sub>2</sub> and 5% CO<sub>2</sub>. Results are shown in Figure 6.

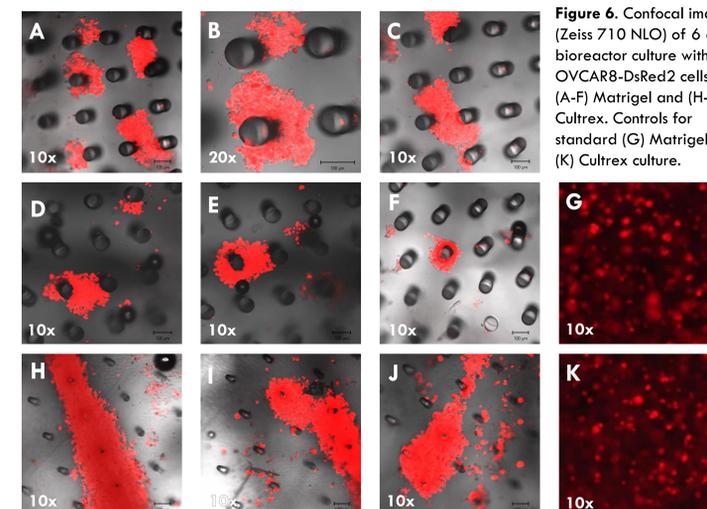


Figure 6. Confocal imaging (Zeiss 710 NLO) of 6 day bioreactor culture with OVCAR8-DsRed2 cells in (A-F) Matrigel and (H-J) Cultrex. Controls for standard (G) Matrigel and (K) Cultrex culture.

## Conclusions & Future Work

- A bioreactor system was successfully designed and constructed for use in 3D cell culture. Hydrogel crosslinking and O<sub>2</sub> gradient characterization presented the largest obstacles.
- Bioreactor culture revealed three dimensional growth around the SiHy pillars, perhaps indicative of oxygen-induced cell migration.
- Future work will involve:
  - improved modeling of oxygen tension through SiHy membrane
  - immunohistochemical gradient studies using pimonidazole
  - gene expression studies comparing cells cultured in our system to other 2D and 3D environments (i.e. spheroids and Matrigel)
  - adaptation for high throughput applications in studies of drug development and multidrug resistance

## Acknowledgements

I sincerely thank my mentors, Tom Pohida and Dr. Michael Gottesman for their extraordinary support and guidance. The culture system was designed and constructed at CIT/DCB. 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 for providing us with Cultrex, and the IRTA and BESIP programs for funding this research.