

Microfabricated PDMS vessel mimetics for cancer cell culture in 6-well plates

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Background & Introduction

The lack of vasculature in 3-D cell culture poses a significant barrier to mimicking *in vivo* Oxygen gradients :

2-D cell culture

- lacks vasculature to mimic 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

3-D cell culture

- contain matrices and scaffolding
- commonly mimic static, short-term conditions
- cell growth and migration into scaffolds limited by O₂ and nutrient transport

Aim 1:

Engineer a microstructure array to simulate vascularization of 3-D cell culture by mimicking O₂ perfusion from blood vessels via microfabricated pillar structures of Polydimethylsiloxane (PDMS)

Aim 2:

Develop a system which will establish an O₂ gradient across multiple 6-well plates, allowing for high-throughput multiplex screening of cell growth patterns.

Aim 3:

Evaluate effect of O₂ gradients on growth patterns of cancer 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 cell growth, we hypothesized that using microfabricated pillars to mimic vasculature would be more reflective of the *in vivo* tumor environment.

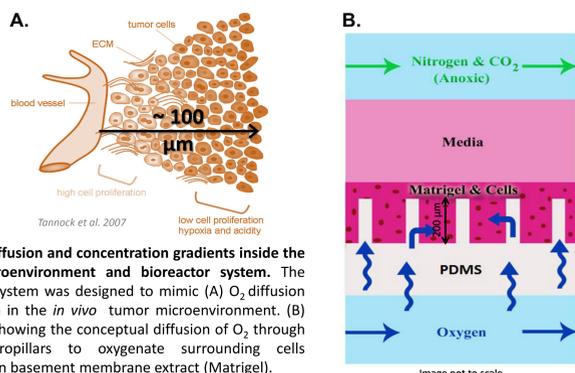


Fig. 1. O₂ diffusion and concentration gradients inside the tumor microenvironment and bioreactor system. The bioreactor system was designed to mimic (A) O₂ diffusion and hypoxia in the *in vivo* tumor microenvironment. (B) Schematic showing the conceptual diffusion of O₂ through PDMS micropillars to oxygenate surrounding cells suspended in basement membrane extract (Matrigel).

Single-Well System

The previous generation bioreactor system provided limited throughput due to a single culture well. A silicone hydrogel membrane was sandwiched in between the upper and lower gas chambers to establish an O₂ gradient.

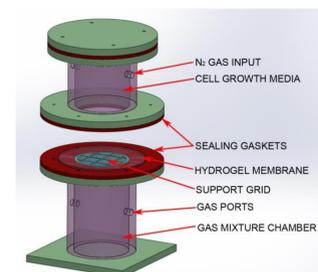


Fig. 2. SolidWorks model of the custom single-well 3D cell culture bioreactor system. Bioreactor with mixed gas flowmeter input. The two flanged components sandwich the fabricated Silicone Hydrogel micropillar membrane, with cells and Matrigel loaded on top before adding media.

PDMS Pillar Microfabrication

Our approach utilizes PDMS as the membrane material. PDMS has proved to be far more durable than the silicone hydrogen used in the single-well system. PDMS was cast into a membrane with high aspect ratio micropillar structures, and incorporated into the bioreactor culture system to deliver O₂ through the micropillars into an otherwise hypoxic culture volume.

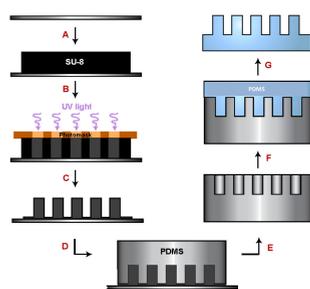


Fig. 3. Process flow for PDMS fabrication. (A) Silicone wafer treatment, photoresist coat, and soft bake. (B) Exposure and post-exposure bake. (C) SU-8 master developed, hard-baked and silanized. (D) PDMS well casting. (E) PDMS well de-molding. (F) PDMS pillar casting (G) PDMS pillar de-molding.

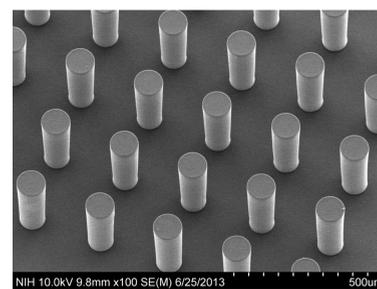


Fig. 4. Scanning electron microscope (SEM) image of PDMS micropillars. Pillars are cylindrical, 100 μm in diameter and 200 μm tall. They are spaced approximately 100 μm apart.

Multi-Well Design and Construction

In order to increase the throughput of our experiments, we designed a bioreactor system that would accommodate multiple multi-well plates, using a custom-fabricated 6-well plate as our prototype. The 6-well format allows for simultaneous testing of multiple pillar geometries and culture conditions. This O₂ gradient system enables the user to easily remove culture plates for media exchange and imaging.

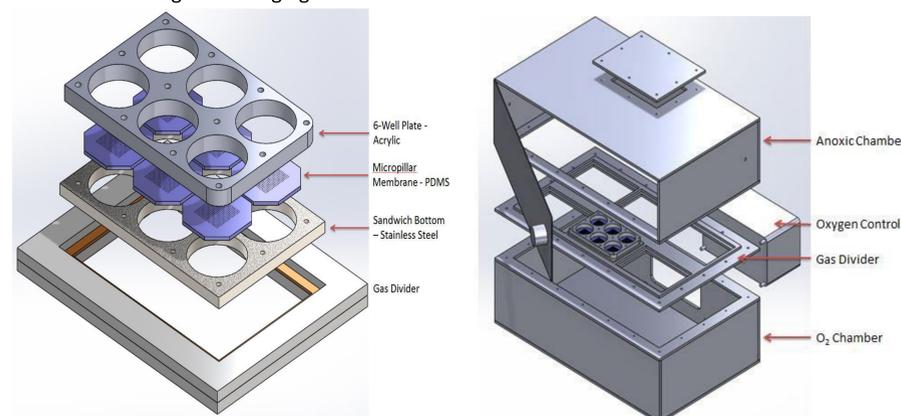


Fig. 5. SolidWorks image of bioreactor 6-well plate sandwich. Pillared 6-well membranes are clamped in between an acrylic top piece and a stainless steel clamp, held together by screws. This sandwich fits onto a gas divider which separates the O₂ condition from the anoxic condition

Fig. 6. SolidWorks image of O₂ gradient chamber. The top chamber houses an anoxic gas mixture. The bottom chamber is maintained at a user specified oxygen condition via the oxygen controller. Between the chambers is a gas divider shelf which can hold six 6-well plate sandwiches.

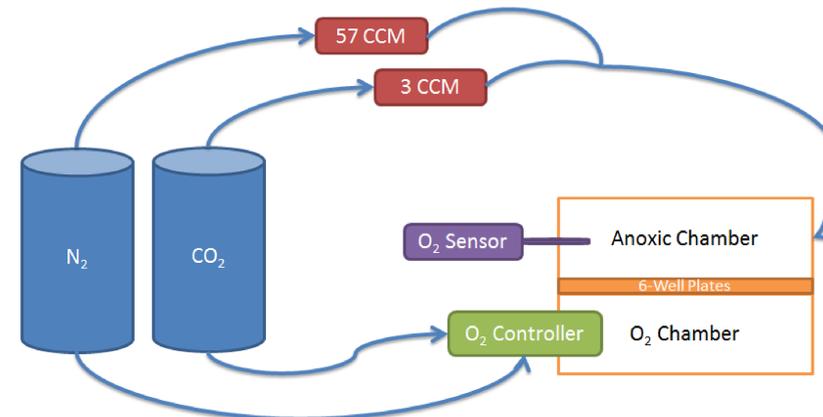


Fig. 7. Diagram of O₂ gradient experimental setup. Nitrogen and Carbon Dioxide gas lines are connected to flow meters to achieve a 95% N₂/ 5% CO₂ mix in the Anoxic Chamber. The lines also feed the Oxygen Controller (ProOx C21, Biospherix) which regulates the percent O₂ in the bottom chamber. A FOSPOR Oxygen Sensor (NeoFox, Ocean Optics) is placed in the top chamber to monitor the anoxic condition throughout the experiment.

Bioreactor Tissue Culture

We set up an experiment to discover how the O₂ gradient affects cell growth patterns. We set up two bioreactor 6-well plate sandwiches, each with one well of 100 μm diameter pillars containing cells suspended in Matrigel. One was placed in a standard tissue culture incubator with 21% O₂, while the other was placed in the O₂ gradient chamber with 8% O₂ condition in the bottom chamber. While the cells in 21% O₂ are evenly spread with little to no clustering, some of the cells exposed to the O₂ gradient are clustered around pillars.

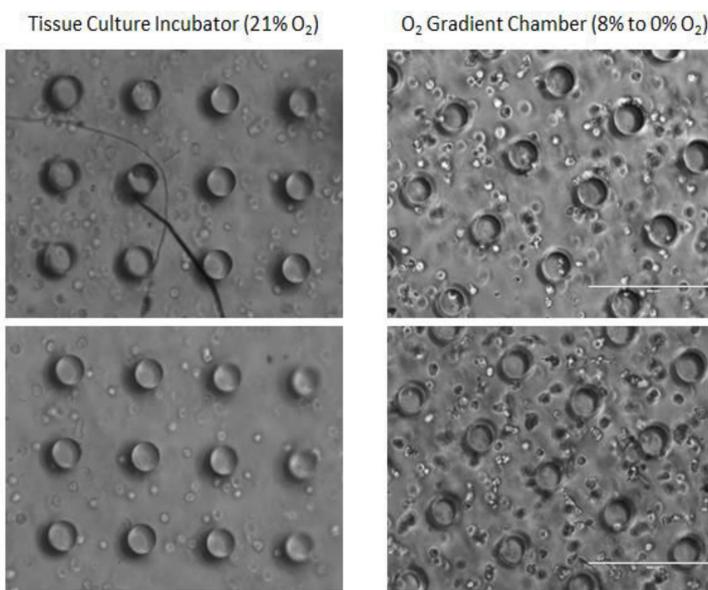


Fig. 8. Bioreactor cell growth with and without O₂ gradient. OVCAR8-DsRed2 cells were cultured for 4 days in 1.5 mL Matrigel on top of a PDMS membrane with pillars. One set was cultured at 21% Oxygen, while the other was cultured in the Bioreactor with 8% Oxygen in the lower chamber. A bright field microscope at 10x magnification was used to take the images.

Conclusions and Future Work

Our 6-well plate compatible bioreactor offers many advantages over the previous single-well version:

- Fabrication of PDMS pillars is far more consistent than silicone hydrogel process. PDMS is less prone to shearing during fabrication.
- O₂ gradient chamber makes it easy to remove culture plate for media exchange and intermediate imaging.
- 6-well plate bioreactor format allows for simultaneous measurement of multiple culture conditions and experimental replicates.

Future work will focus on characterizing the effects of several variables such as different cell types, media, pillar geometries, oxygen gradients, as well as bioreactor integration with real-time cell imaging methodologies.

References

Ashley A. Jaeger, Chandan K. Das, Nicole Y. Morgan, Randall H. Pursley, Philip G. McQueen, Matthew D. Hall, Thomas J. Pohida, Michael M. Gottesman, *Microfabricated polymeric vessel mimetics for 3-D cancer cell culture*, Biomaterials, Available online 30 July 2013, ISSN 0142-9612, <http://dx.doi.org/10.1016/j.biomaterials.2013.07.013>.

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