

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:

2-D cell culture

- lacks vasculature 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 (SiHy).

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.

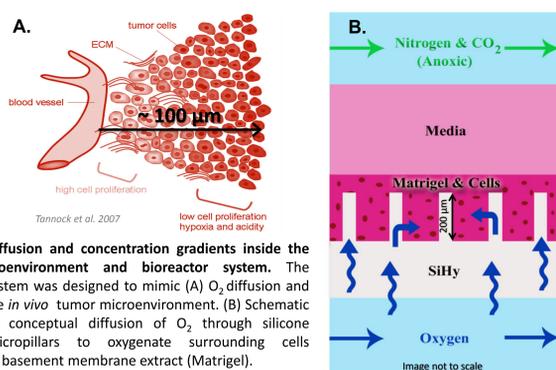


Fig. 1. O₂ diffusion and concentration gradients inside the tumor microenvironment and bioreactor system. The bioreactor system was designed to mimic (A) O₂ diffusion and hypoxia in the *in vivo* tumor microenvironment. (B) Schematic showing the conceptual diffusion of O₂ through silicone hydrogel micropillars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

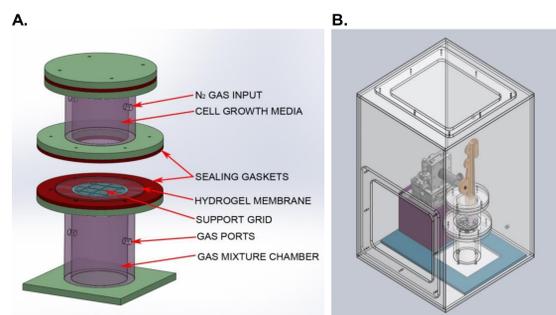


Fig. 2. SolidWorks model of the constructed 3D cell culture system. (A) Bioreactor with mixed gas flowmeter input. The two flanged components sandwich the fabricated SiHy micropillar membrane, with cells and Matrigel loaded on top before adding media. (B) Airtight container to house the culture system with a micrometer-driven stage for precise measurements of O₂ and pH in the culture medium.

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 methacrylate chemistry, and incorporated into the bioreactor culture system to deliver O₂ through the hydrogel pillars into an otherwise hypoxic surrounding media.

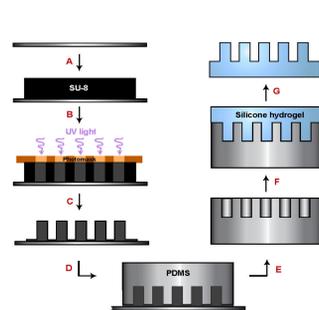


Fig. 3. Process flow for silicone hydrogel (SiHy) fabrication. (A) Silicone wafer treatment, photoresist coat, and soft bake. (B) Exposure and post-exposure bake. (C) SU-8 master developed, hard-baked and silanized. (D) PDMS casting. (E) Demolding. (F) SiHy casting and photopolymerization. (G) SiHy membrane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

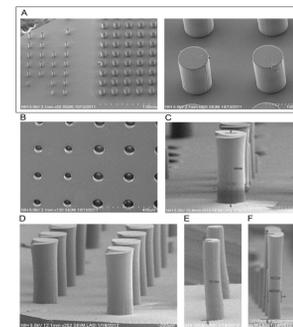


Fig. 4. Scanning electron microscope (SEM) images of micropillars at different processing steps of silicone hydrogel fabrication. (A) SU-8 micropillar master mold. (B) Negative PDMS mold replicated from SU-8 master. (C-F) SiHy micropillars cast from PDMS molds. Micropillar diameter ranged from 25 – 100 μm with height of 200 – 250 μm, achieving an aspect ratio of 7.5-8.

Modeling the Microenvironment

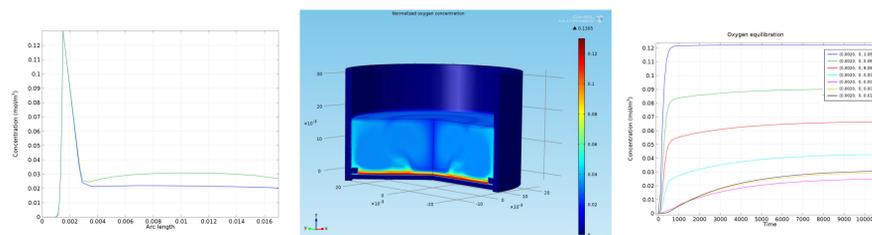


Fig. 5. Spatial variation in normalized equilibrium oxygen concentration. Arc length is the height above the silicone hydrogel (mm), with the surface at 0.002mm. The blue curve is at the center of the bioreactor, green curve is offset by 2mm from the center. Note the relatively flat and non-zero value in the water outside the agar layer, about 10-20% of the max O₂ concentration.

Fig. 6. Normalized whole-bioreactor calculation of convective flow. Natural external convection outside the bioreactor was assumed at 37°C, with forced gas convection at 34°C below the hydrogel and above the liquid. Maximum flow velocities were on the order of 20-50 μm/s, with conductive flow predominantly up the bioreactor edges, and down the center. Plugging the stable flow field into a simplified diffusion model gave gradient profiles that were qualitatively and quantitatively similar to what was measured with the oxygen probe.

Fig. 7. Preliminary modeling of O₂ equilibration time scales. Profiles are 0.002 mm off center, at different heights above the SiHy surface. Early results show a relatively long time scale for equilibration at 5 and 10 mm away from the hydrogel surface (just stabilizing after 2.5 h), which is comparable to prior oxygen probe measurements in the bioreactor.

Oxygen Gradient Analysis

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 hypoxia gradients within tumor spheroids cultured in the bioreactor, the hypoxia marker Hypoxyprobe-1 (Pimonidazole HCl) was utilized for immunohistochemical analysis (Fig. 9).

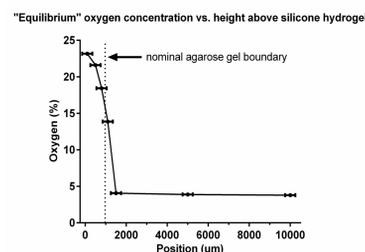


Fig. 8. O₂ tension gradient as a function of distance away from a hydrogel sheet. Oxygenation was ~21% in the bottom chamber of the bioreactor, while the top chamber was flooded with 95% N₂ and 5% CO₂. Recordings were completed at various distances from a flat SiHy surface with a 0.3% agarose gel layer using the micrometer stage set-up as seen in Fig. 2B. Error bars indicate potential positional error related to the 500 μm outer diameter of the fiber-optic O₂ probe.

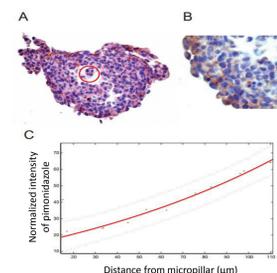


Fig. 9. *In vivo*-like O₂ gradient exists within tumor organoids cultured in the bioreactor system. (A) Pimonidazole staining of a tumor organoid removed from a SiHy micropillar (location circled) after 7 d culture with the bottom chamber maintained at 3% O₂. (B) Image highlighting pimonidazole staining intensity gradient used to generate an (C) analysis of staining intensity (hypoxia) as a function of distance from the pillar (tumor organoid center).

Bioreactor Tissue Culture

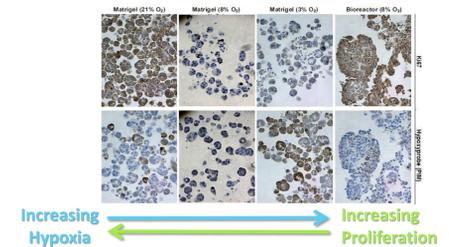


Fig. 10. Tumor hypoxia and cell proliferation status in 3-D cell culture at different O₂ concentrations. OVCAR8-DsRed2 cells were cultured for 7 d at 3, 8 and 21% O₂ in Matrigel and with 8% O₂ in the lower chamber of the bioreactor culture system. Cell proliferation and hypoxia staining were performed with Ki67 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.

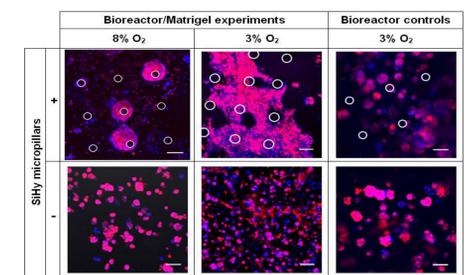


Fig. 11. 3-D cultures of OVCAR8-DsRed2 cells oxygenated via micropillars generate distinct culture geometries. The positions of the micropillars are shown as white circles. Confocal images of 7 d bioreactor cultures in Matrigel with the upper bioreactor chamber maintained at 0% O₂ (95% N₂, 5% CO₂) and lower bioreactor chambers maintained at 3% (top center) and 8% O₂ (top left). Control cultures grown in Matrigel for 7 d at 3% (bottom center) and 8% O₂ (bottom left) show smaller spheroidal morphologies. Another type of control experiment involved culturing cells in the bioreactor in the absence of an O₂ gradient (top right). Both chambers were kept at 3% O₂ for 7 d to serve as a bioreactor control. A second bioreactor control experiment involved an O₂ gradient, but used a flat SiHy membrane (bottom right). The top and bottom chambers were kept at 0% O₂ and 3% O₂, respectively.

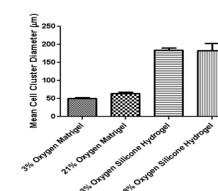


Fig. 12. Cancer cells vascularized by micropillars show increased size distribution. Cultures grown for 7 d in Matrigel or within Matrigel on micropillar membranes in hypoxia (3% and 8%) and normoxia (21%). Images taken with Zeiss Axiovert fluorescent microscope and Axiovision software was used to quantify cell spheroid sizes.

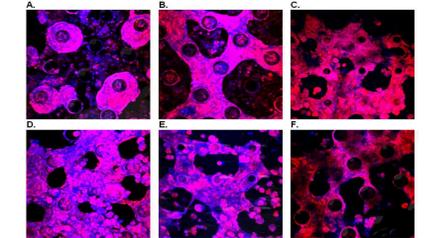
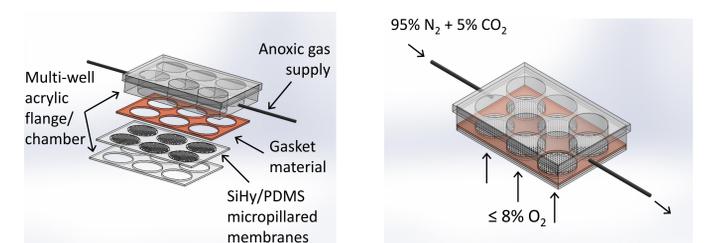


Fig. 13. PDMS bioreactor culture demonstrates unique culture geometries similar to silicone hydrogel micropillars. Maximum intensity projections of confocal z-stack images of OVCAR8-dsRed2 cells (red) with Hoechst stain (blue). Cells were cultured 7 d in the bioreactor system (3% O₂ in bottom chamber) on microfabricated PDMS pillars.

Future Work



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