Recapitulating *in vivo* Spatial Oxygen Distribution in 3D Cancer Spheroids

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Introduction

In vitro cancer models have the potential to give precise control over microenvironmental factors involved in tumor growth. Traditional monolayer culture does not accurately replicate *in vivo* cues, causing differences in cell morphology, gene expression, and signaling. This is in part since oxygen levels in cell culture incubators are vastly different from oxygen concentrations *in vivo*[1-3].

To address these issues, our lab has developed a bioreactor system that can controllably deliver oxygen to cells in 3D culture through an array of microfabricated polydimethylsiloxane (PDMS) pillars, emulating a capillary bed (Fig. 1). This pillared surface forms a partition between two chambers, an upper hypoxic chamber and a lower 3% O_2 chamber mimicking *in vivo* venous pO_2[4]. Preliminary experiments with the bioreactor show significant differences in gene expression from 3D culture controls.

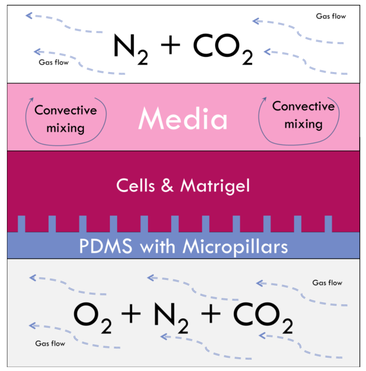


Fig 1. *Schematic of PDMS membrane gradient oxygenation conditions with cancer cells in matrigel and media. Micropillared regions are incorporated into a 6-well plate format.*

Motivation

While the current system produces an oxygen gradient, it does not mimic oxygen delivery from capillaries since the base surrounding the pillars is also oxygen permeable (Fig. 2). To improve on this design, a polymer coating can be added to block oxygen transmission around the pillars and further localize the delivery of oxygen (Fig. 3).

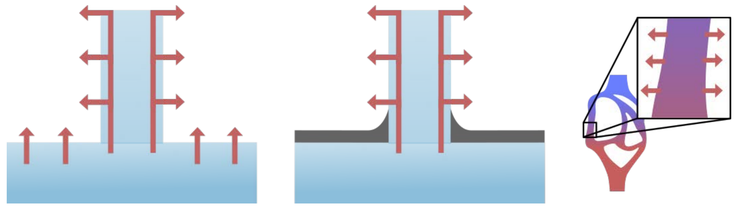


Fig 2. *General comparison of oxygen distributions between current membranes, proposed coating method, and a capillary bed.*

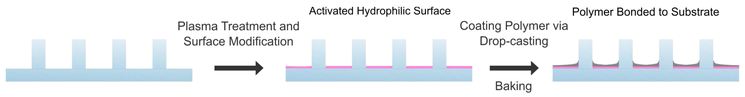


Fig 3. *(Above) Proposed coating method workflow for bonding polymer coating to base of PDMS pillars. (Below) Surface modification methods to test.*

Polymer	Surface Modification	Solvent
TPU	Plasma Treatment	DMSO
TPU	Plasma Treatment + Silane	DMSO
PMMA	Plasma Treatment	Ethyl Acetate
PMMA	Plasma Treatment + Silane	Ethyl Acetate

Coating and Surface Modification

Drop-casting of polymer solutions of thermoplastic polyurethane (TPU) and poly(methyl methacrylate) (PMMA) was found to be the best coating method. Surface modification was also tested with 3-(trimethoxysilyl)propyl methacrylate (TMSPMA), to prevent delamination.

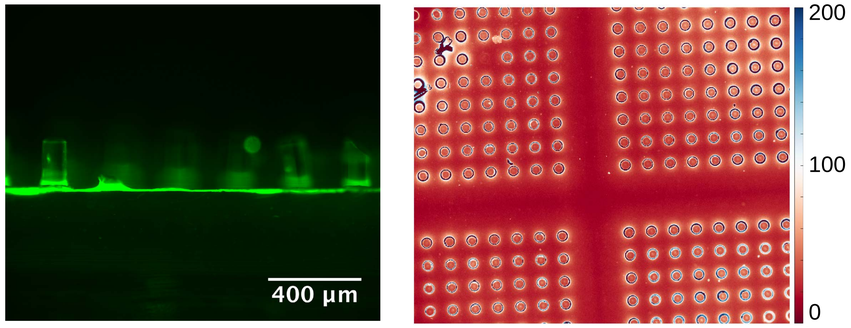


Fig 4. *(Left) Cross section of coated PDMS well. (Right) MATLAB conversion of top-down fluorescence intensity to thicknesses of coated film.*

Fluorescent images of wells coated with dye-loaded polymer were analyzed with MATLAB to approximate thicknesses across wells (Fig. 4). 20 µL of TPU and PMMA was used as the working volume, since larger volumes covered the pillars significantly. TPU-TMSPMA, PMMA-TMSPMA, and PMMA coatings were found to be the most optimal.

Cell Culture

OVCAR8 cells transfected with dsRed were cultured on coated and plain PDMS membranes for one week in the bioreactor, in 3%, and in 21% oxygen, (Fig. 5) and then imaged with both wide-field and confocal microscopy (Fig. 6).

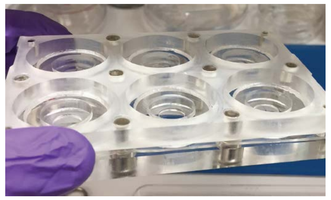


Fig 5. *(Left) Custom 6-well plate for PDMS membranes. (Right) Bioreactor incubator setup. Gradient condition plates placed in center holders*

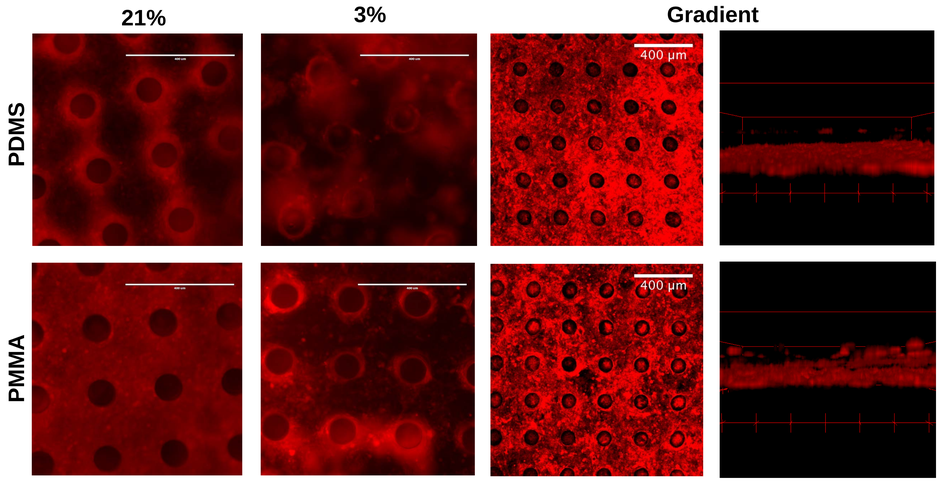


Fig 6. *Comparison of cell growth between wide-field fluorescent images of cells grown on uncoated and PMMA coated wells in 21% and in 3% oxygen controls as well as maximum intensity projections and 3D views of confocal images.*

Oxygen Distribution

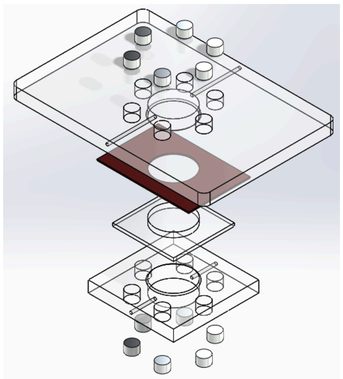
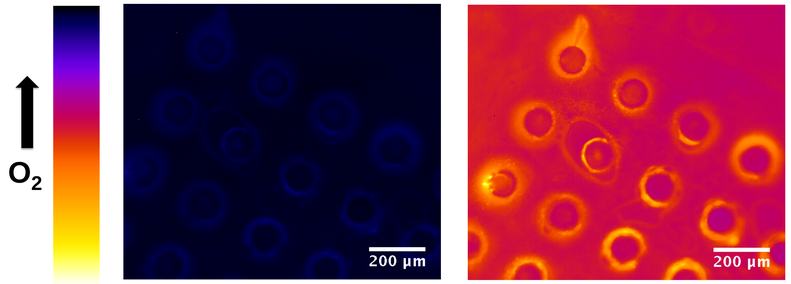


Fig 7. *Exploded view of Solidworks chamber design.*

To measure the spatial distribution of oxygen around the pillars, a microscope-compatible device with separate hypoxic and 21% oxygen chambers (Fig. 7) was designed and fabricated by 3D printing.

An oxygen sensitive dye, tris(2,2′-bi-pyridyl)dichlororuthenium(II) (RTDP), was imaged in a coated well to assess the spatial oxygen distribution over time as a proof-of-concept. Fluorescent images were taken of single wells covered in dye-loaded agarose gel using the custom made chamber (Fig. 8).

Fig 8. *Pseudo-colored images of of RTDP in TPU-TMSPMA well (left) before and (right) after prolonged N_2 flow.*



A simple way to selectively apply an oxygen blocking layer to micropillared membranes was developed, and a device for measuring the resulting spatial variation of oxygen was constructed. It has been shown that these coatings are compatible with 3D culture, and qualitative differences in cellular growth patterns from controls were observed after seven days culture.

Future work includes quantifying cell growth/aggregation in comparison to controls with nuclear staining of coated wells, as well as gene expression analysis of cells grown in these conditions. While the current setup for imaging oxygen distribution could give a rough estimate of oxygen concentrations, fluorescence lifetime imaging and further design improvements of the chamber and will be needed for precise measurements. Notably, this chamber could also be used to image cells under gradient oxygenation conditions in real-time.

[1] Elsdale T, Bard J. Collagen substrata for studies on cell behavior. J Cell Biol. 1972; 54:626–637.

[2] Pampaloni F, Reynaud EG, Stelzer EHK. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Bio. 2007; 8:839–845.

[3] Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. Nat Rev Cancer. 2005; 5:675–688.

[4] Goldman L, Schafer AI., editors Goldman's Cecil Medicine. 24th ed. Philadelphia, PA: Elsevier/Saunders; 2012.

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