MICROFLUIDIC GAS DIFFUSION PLATFORM

Bradley Wendorff – Team Leader
Drew Birrenkott – Communicator
Caleb Durante – BWIG
Jared Ness – BSAC

Professor Brenda Ogle, Ph.D. – Client
Professor Tracy Puccinelli, Ph.D. - Advisor

OVERVIEW

- Background – Microfluidic Devices
- Client requirements and desired specifications
- Critical analysis of two design elements
  - PDMS diffusion platform
  - Oxygen detection technique
- Current design
- Moving forward

PROBLEM STATEMENT

- Need way to assess cardiac cellular response to hypoxia
- Traditional hypoxia chambers non-ideal
  - Slow, Large & space-filling, $$$
- TASK: Develop and validate a next-generation, microfluidic-based hypoxia chamber to facilitate studies involving oxidative stress, ischemia, and reactive oxygen species (ROS)-mediated cellular pathways.

MICROFLUIDIC DEVICES

- Flexible polymer matrix (PDMS)
- Fabrication Process
  - Molded over master template
  - Channels cross-linked to glass
  - Cells seeded in fluid filled channels
- Applications of microfluidics
  - Printer industry
  - Study of microbial behavior
  - Study of cellular behavior**

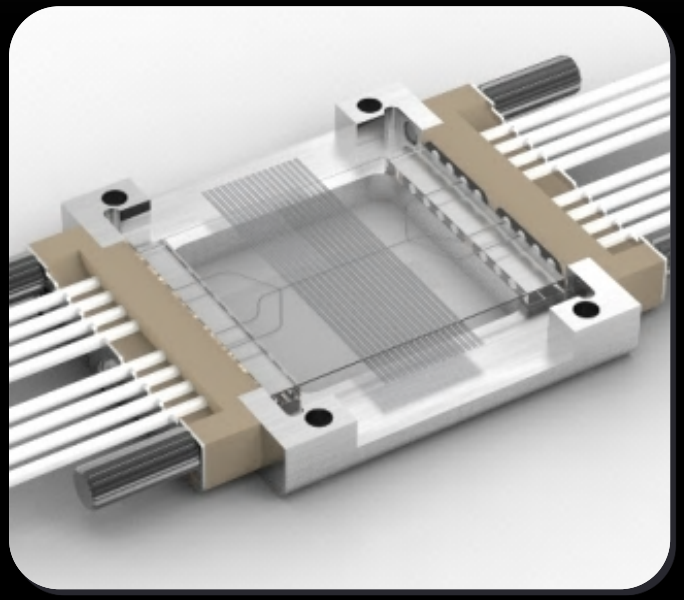

Figure 1: PDMS platform connected to fluid lines (Image taken from www.dolomite.com)

DESIGN SPECIFICATIONS

- Oxygen gradient range: 21% - 1%
- Cannot interfere with cell culture
- Master mold reusable
- PDMS device one-time use
- Biocompatible, non-cytoxic materials only
- Operate at 37˚C in a 5% CO2 incubator
- Channels: 250μm - 500μm tall x 250μm – 750μm wide

PLATFORM CHANNEL LAYOUT

- Design 1 – Parallel Flow
  - Gas flow at a constant rate
  - Flow release based on pulsating solenoid manifold
  - Diffusion of O_2 and N_2 into micro-wells
  - Costly

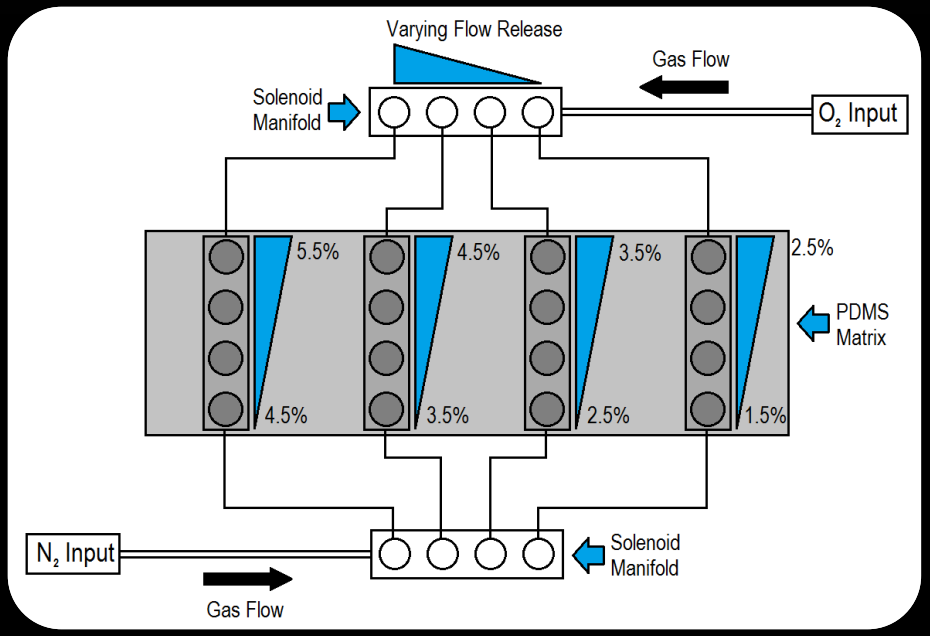

Figure 2: Top view schematic of parallel flow design.

PLATFORM CHANNEL LAYOUT

- Design 2 – “Two-Channel”
  - O_2 and N_2 flow into gas channels
  - O_2 gradient forms across channels
  - Relatively inexpensive and simple

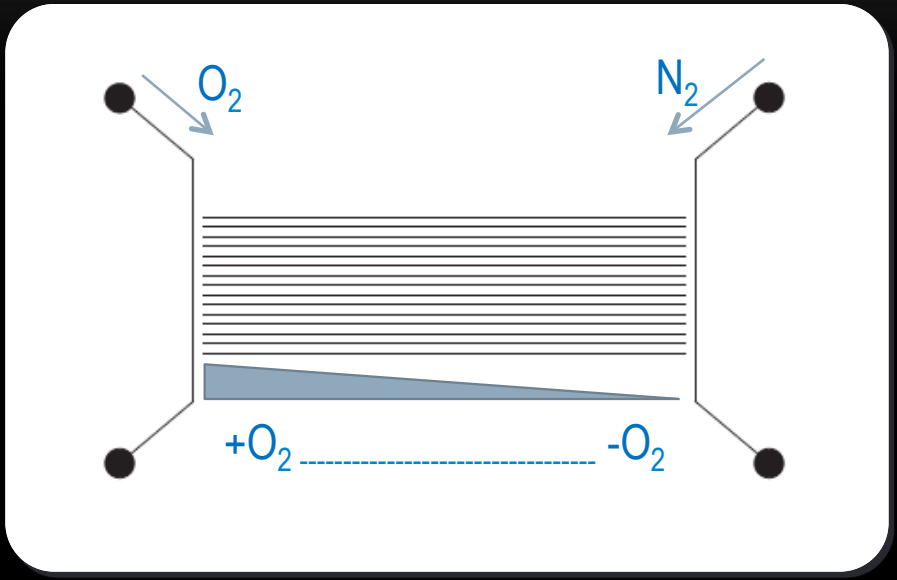

Figure 3: Two channel design concept (Based on Li, et. Al 2011).

PLATFORM CHANNEL LAYOUT

- Design 3 – “Oxygenator”
  - Requires precise microfluidic construction
  - Concentrations halved at each node
  - Can develop full spectrum gradient (0-100%)
  - Cell platform situated above R_{out}

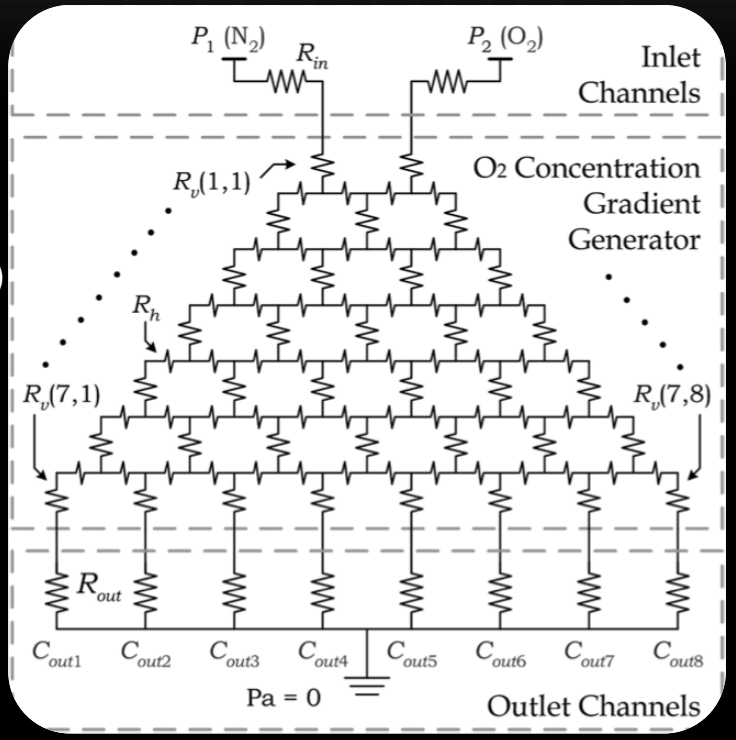

Figure 4: O_2 gradient C_{out1}-C_{out8}
0% - 14.2% - 28.49% - 42.82% - 57.18% - 71.53% - 85.81% - 100%
(Lam, et. Al 2009)

CHANNEL DESIGN MATRIX

Platform Design				
Factors	Weight	Rating (1-10)		
		Parallel Flow	Two Channel	Oxygenator
Ease of production	0.25	4	9	2
Span of gradient range	0.20	4	7	9
Cell-culture isolation	0.15	8	5	6
Gradient Control	0.25	8	4.5	2
Cost	0.15	1	6	7
TOTAL	1	5.15	6.425	4.75

GAS DETECTION METHODS

- Thin sensor Film
  - Layer of Chemo-fluorescent indicator molecule
  - Embedded in porous matrix
  - Quenched by O_2
  - Concentration based on fluorescent intensity
- Sensor matrix replaced after each experiment

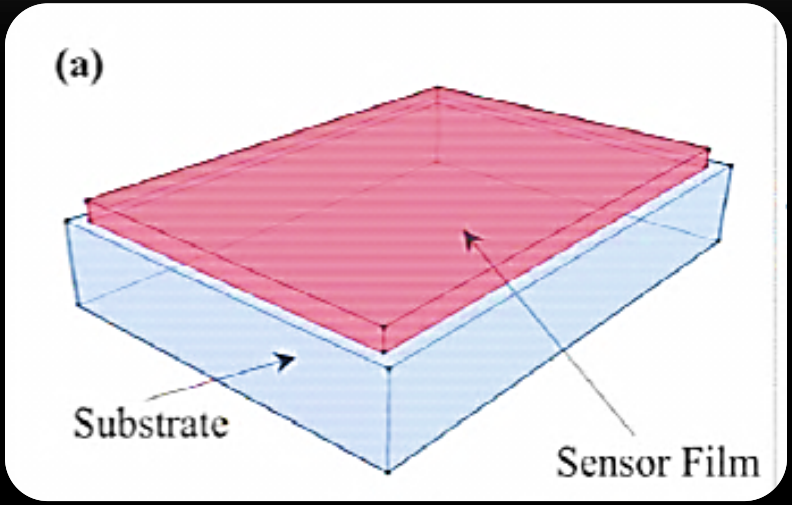

Figure 5: Representation of the thin sensor film design (Grist, et. Al 2010).

GAS DETECTION METHODS

- Fluorescent microparticles
  - Suspended in cell culture media
  - Coated in PDMS
  - Fluorescent intensity-based

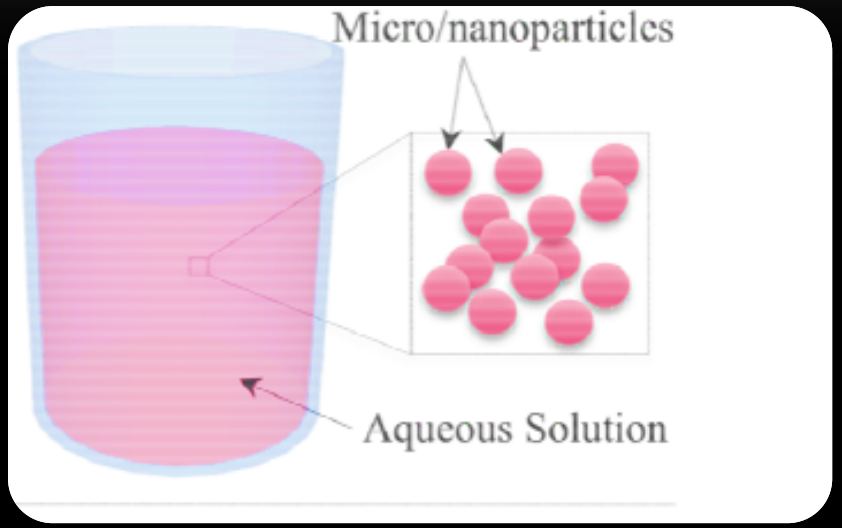

Figure 6: Representation of PDMS coated microparticles in solution (Grist, et. Al 2010).

GAS DETECTION METHODS

- O_2 microelectrode sensor
  - Gives discrete measurement for one location
  - O_2 reduction produces voltage
  - O_2 is consumed
    - Affects concentration

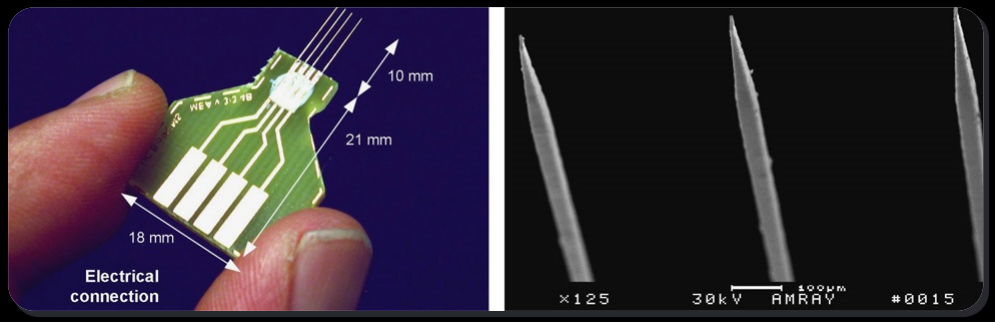

Figure 7: Dissolved oxygen microelectrode (Left) and dissolved oxygen sensing tips (Right) (Lim, et. Al 2009).

GAS DETECTION DESIGN MATRIX

Method of Monitoring Oxygen				
Factors	Weight	Rating (1-10)		
		Thin Sensor Film	Fluorescent Particles	O_2 Probe
Accuracy	0.30	7	8	2
Cost	0.15	4	5	3
Ease of Use	0.25	7	4	7
Biocompatibility	0.30	8	6	8
TOTAL	1.00	6.85	5.95	5.2

PRELIMINARY DESIGN

Figure 8: SolidWorks rendition of the 2 channel design (Based on Li, et. Al 2011).

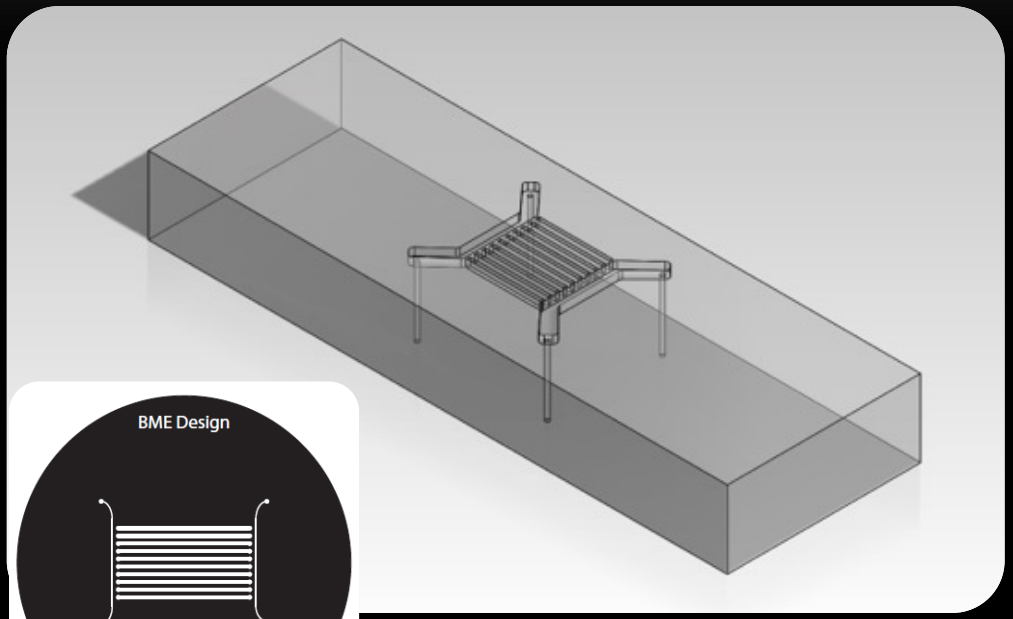

Figure 9: Two channel design photo mask (Based on Li, et. Al 2011).

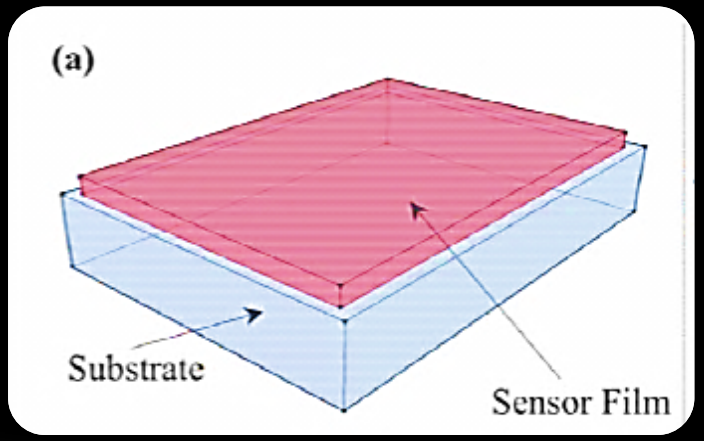

Figure 10: Representation of the thin sensor film design (Grist, et. Al 2010).

FUTURE WORK

- Chemical safety training
- Construct 2-channel device
- Calibrate florescence detector
- Integrate all design components

ACKNOWLEDGEMENTS

- Professor Brenda Ogle
- Professor Tracy Puccinelli
- Professor John Puccinelli
- Brian Freeman

REFERENCES

Beebe D, M. G., Walker G. "Physics and Application of Microfluidics in Biology." Annual Review of Biomedical Engineering **4**: 261-286.

Birgit Ungerböck, G. M., Verena Charwat, Peter Ertl, Torsten Mayr (2010). "Oxygen imaging in microfluidic devices with optical sensors applying color cameras." Procedia Engineeering **5**: 456-459.

Eddington, e. a. (2009). "Modulating Temporal and Spatial Oxygenation over Adherent Cellular Cultures." PLoS ONE **4**(9).

Grist, S. C., L. Cheung K. (2010). "Optical Oxygen Sensors for Applications in Microfluidic Cell Culture." Sensors **10**: 9286-9316.

Lam R, K. M., Thorsen T. (2009). "Culturing Aerobic and Anaerobic Bacteria and Mammalian Cells with a Microfluidic Differential Oxygenator." Anal. Chem. **81**: 5918-5924.

Li N., Luo C.X., Zhu X.J., Chen Y., Qi O.Y., Zhou L.P. (2011). "Microfluidic generation and dynamically switching of oxygen gradients applied to the observation of cell aerotactic behaviour." Microelectric Engineering 88(8): 1698-1701.

Lo J., S. E., Eddington D., (2010). "Oxygen Grandients for Open Well Cellular Cultures via Microfluidic Substrates." NIH Public Access: 15.

Sin, A. C., K. Jamil, M. Kostov, Y. Rao, G. Shuler, M. (2004). "The Design and Fabrication of Three-Chamber Microscale Cell Culture Analog Devices with Integrated Dissolved Oxygen Sensors." Biotechnol. Prog. **20**(1): 338-345.

Ungerbock, B. M., G. Charwat, V. Ertl, P. Mayr, T. (2010). "Oxygen imaging in microfluidic devices with optical sensors applying color cameras." Elsevier **5**: 456-459.