GENERATION OF AN ACCESSIBLE AND VERSATILE MICRO-HYPOXIA CHAMBER

Client: Professor Brenda Ogle Advisor: Dr. John Puccinelli

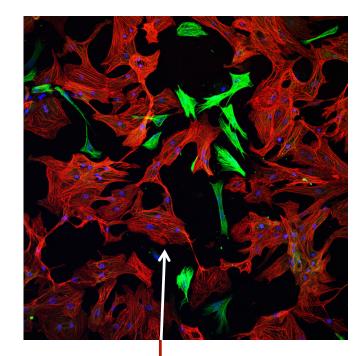
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Overview

- Background
- Problem Identification and Statement
- Product Design Specifications
- Previous Work, Current Technology
- Design Alternatives
- Design Matrix
- Future Work
- Acknowledgements and Questions

Background: Hypoxia and the Heart

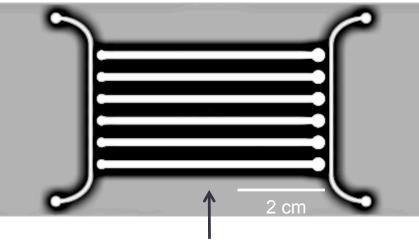
- Heart attack is the number one cause of death in the United States
- A proposed treatment for heart attacks is stem cell fusion with damaged tissue. This occurs primarily in hypoxic conditions



A cardiomyocyte¹

Hypoxia: phenomena where regions of tissue are deprived of necessary oxygen

Background: Microfluidics in Hypoxia



Microfluidic device capable of maintaining concentration gradient

- Devices made of PDMS, which is gas permeable
- Microfluidic devices can easily create oxygen gradients
- Allows use of limited cells and reagent: low cost

Problem Statement

 Microfluidic device has been developed to mimic hypoxia in heart

 Goal is to create oxygen sensor to monitor oxygen gradient in real time in device

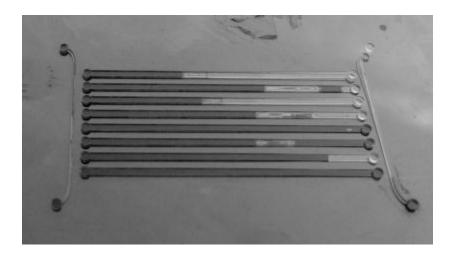
Product Design Specifications

- Detect O₂ from 1% to 20% concentrations with 2% accuracy
- Does not impede microscopy
- Small fabrication time
- Stable in incubator
- Under 50 grams
- Integrate with current PDMS device
- Not cytotoxic to cells of interest
- Projected Cost \$500

Previous Work

Spring 2012

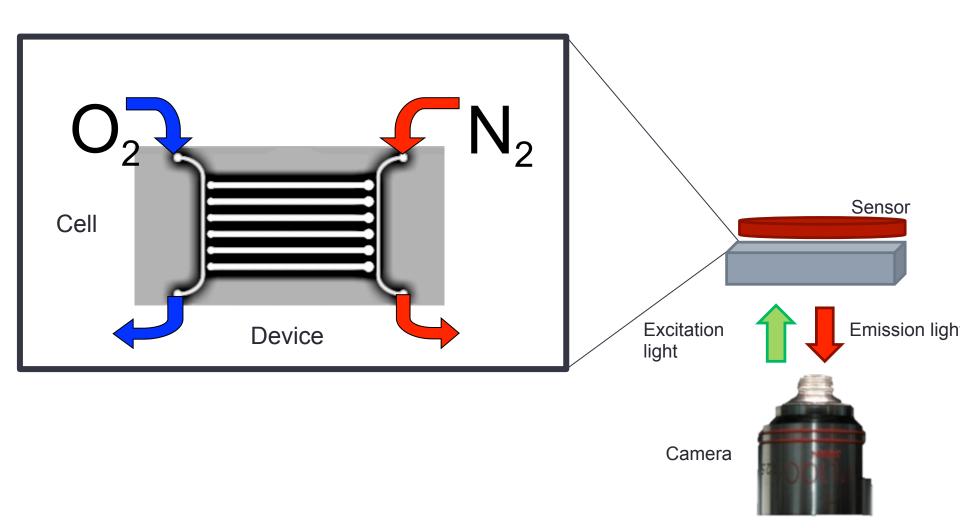
Master Template



Fall 2012

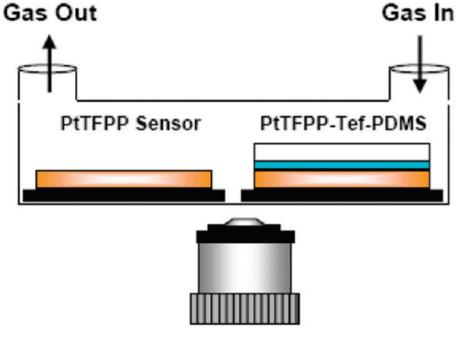
- PtOEPK sensor
 - Fluorescent porphyrine
 molecule
 - 96 well plate
 - Testing in progress

Previous Work: Operational Theory



Current Technology

- University of Wisconsin PtTFPP Teff PDMS sensor
- University of Michigan thin sensor film



Model of PtTFPP – Teff – PDMA sensor²

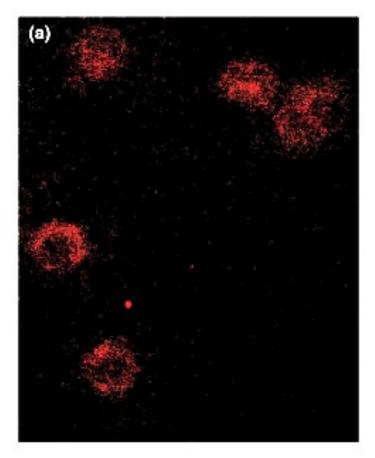
Design Alternative: PtOEPK

Advantages

- High O₂ Sensitivity
- No leaching effects
- Repeatable
- Low Cost

Disadvantages

- Biological nature leads to variability
- Non-standard emission wavelength



PtOEPK in use as an optical sensor³

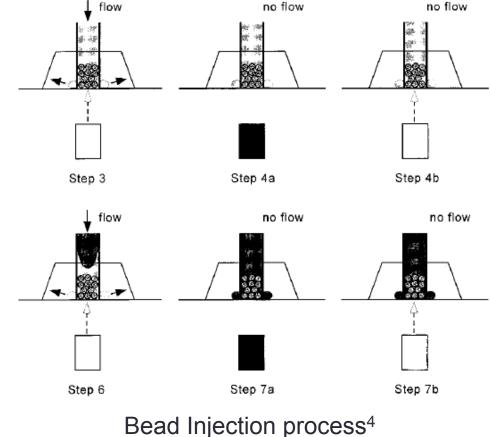
Design Alternative: Bead Injection Spectroscopy

Advantages

- O₂ dependent quenching of the phosphorescence of a Pt – porphyrin complex immobilized
- Fast response: minutes
- Renewable sensor surface

<u>Disadvantages</u>

- Consumes O₂
- Can be easily affected by surrounding media
- Limited success in previous literature



Design Alternative: Electrical Sensor

Advantages

- Has strong basis in fiber optics
- Accurate and sensitive
- Produces discrete quantitative data
- Potentially reusable



Disadvantages

- Requires multiple detector units
- Introduces new system (electrical vs. biological)
- Larger initial investment

Fiber optic sensors⁵

Design Matrix

Category	PtOEPK	Bead Injection Spectroscopy	Electrical Sensor
Sensitivity (25%)	4	2	4
Accuracy (25%)	3	2	4
Integration (15%)	4	3	2
Ease of Fabrication (10%)	4	5	1
Response Time (10%)	4	4	3
Cost (15%)	3	3	2
Total	72	56	60

Future Work and Expectations

• Aim one:

- Purchase material
- Testing and calibration curve
- Aim two:
 - Design and integrate the sensor onto the device
 - Calibration experiment with cells
 - Protocol writing

Future Work



Sensor made 96-well plate

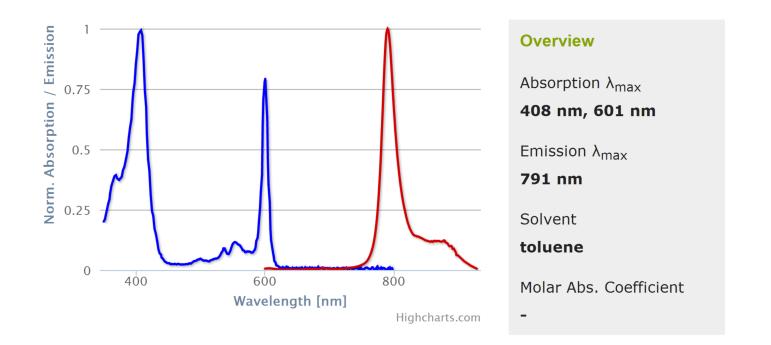
Secure the sensor plate under hypoxia chamber

Adjust oxygen concentration (20% to 5%)

Measure the intensity of light

Response

 The PtOEPK based sensor responds to excitation light of wavelengths 408 & 601 nm and has emission at a non-standard wavelength of 791 nm



Acknowledgements

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- Max Salick
- Dr. Randolph Ashton
- Drew Birrenkott
- Previous groups

References

[1] *Health Age*. <http://www.thehealthage.com/2012/04/scar-tissue-can-be-transformed-into-beating-hearts/ cardiomyocyte/>

[2]Thomas, P. C., Halter, M., Tona, A., Raghavan, S. R., Plant, A. L., & Forry, S. P. (2009). A noninvasive thin film sensor for monitoring oxygen tension during in vitro cell culture. Analytical chemistry, 81(22), 9239-9246.

[3] Sarah M Buck, Yong-Eun Lee Koo, Ed Park, Hao Xu, Martin A Philbert, Murphy A Brasuel, Raoul Kopelman, Optochemical nanosensor PEBBLEs: photonic explorers for bioanalysis with biologically localized embedding, *Current Opinion in Chemical Biology*, Volume 8, Issue 5, October 2004, Pages 540-546, ISSN 1367-5931, 10.1016/j.cbpa.2004.08.011. (http://www.sciencedirect.com/science/article/pii/S1367593104001097)

[4] Detection of oxygen consumption of cultured adherent cells by bead injection spectroscopy - American Chemical Society. doi: - 10.1021/ac990712b

[5] Ocean Optics. <oceanoptics.com>

Grist, Samantha et al., Optical Oxygen Sensors for Applications in Microfluidic Cell Culture, Sensors 2010, 10, 9286-9316; doi: 10.3390/s101009286

Lo, Joe et al., Oxygen Gradients for Open Well Cellular Cultures via Microfluidic Substrates, Lab Chip. 2010 September 21; 10(18): 2394–2401. doi:10.1039/c004660d.

Wise, R. R., & Naylor, A. W. (1985). Calibration and use of a clark-type oxygen electrode from 5 to 45°C. Analytical Biochemistry, 146(1), 260-264. doi: 10.1016/0003-2697(85)90424-5

QUESTIONS?