DEVELOPING AN OXYGEN DETECTION DEVICE FOR A MICROFLUIDIC-BASED HYPOXIA CHAMBER

TEAM: Matthew Zanotelli, Chelsea Bledsoe, Karl Kabarowski, Evan Lange
CLIENT: Professor Brenda Ogle, PhD, Department of Biomedical Engineering – University of Wisconsin - Madison
ADVISOR: Professor Randolph Ashton, PhD, Department of Biomedical Engineering – University of Wisconsin - Madison

Abstract

Heart attacks are the number one killer of both men and women in the United States. When a heart attack restricts the oxygen-rich blood flow to cardiac cells, the cells die and cannot be re-grown. A proposed treatment for reversing this damage is the fusion of stem cells into the damaged tissue. It is shown that this fusion is more likely to occur under hypoxic conditions, where the cells are introduced to low levels of oxygen. Such conditions are mimicked in in-vitro microfluidic-based hypoxia chambers, which create an oxygen gradient across microfluidic channels. An important component in these devices is detecting accurate oxygen concentrations within the channels to ensure that the cells are truly under hypoxic conditions. Thus, this design project focuses on developing an oxygen detection method for use in the microfluidic hypoxia chamber that was created in the spring of 2012 by the previous design team. The oxygen detection method alternatives consist of three different formats and two indicators. The formats for the sensor include thin-film strips, micro/nanoparticles, and water-soluble macroparticles. Additionally, the indicator alternatives are ruthenium-based and metalloporphyrin-based. After analyzing these designs, a final design of a thin-film sensor with a metalloporphyrin-based indicator was determined. The thin-film sensor will consist of platinum (II) octaethylporphyrinketone (PtOEPK) in a polystyrene encapsulation matrix. The thin-film sensors were fabricated and tested in 96-well plate and imaged for fluorescent intensity using a confocal microscope. Intensity levels at atmospheric conditions (21% O_2) were measured and recorded. The device demonstrated sensitivity to oxygen and provides a viable option for oxygen detection and monitoring within a microfluidic construct.

Background

Motivation

Myocardial Infarctions (heart attacks)
- Responsible for 1 in 4 deaths [1]
- Result in cell death

Proposed Treatment
- Stem cell fusion to produce new cells
- Fusion more likely under hypoxic conditions [2]

Microfluidic Devices
- Micro-scale fluid mechanisms
- Small devices with channels
- Used to make hypoxia chambers


Figure 1. The microfluidic device designed by the previous Ogle design team.

Current Devices

Research Devices:
- Oxygen detection for specific microfluidic devices
- Designed for specific labs

Commercial Devices:
- Large-scale detection
  - •Thin-film sensors
  - •Electrodes
- None for oxygen detection in microfluidic devices

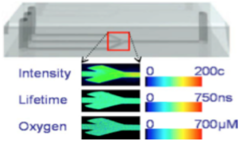


Figure 2. Illustration of fluorescence intensity and lifetime imaging in microfluidic devices [3].

Problem Statement

- Pressing need to understand impact of hypoxic stress on cells
- Use of microfluidic devices to generate hypoxic environments
- Devices will be used to study:
  - •Oxidative stress
  - •Ischemia
  - •Reactive oxygen species – mediated cellular pathways
- Previous semester: Produced functioning microfluidic-based hypoxia chamber
- **This semester**: Develop accurate oxygen detection mechanism for the device

Design Criteria

- Detect oxygen concentrations from 1% to 21%
- High level of repeatability
- Low cost
- Function within a range of +/- 2 to 3% oxygen concentration
- Last through one experiment (up to 2 weeks)
- Operation in an incubator (37°C and 5% CO_2)
- Exposure to fluorescence

Final Design

Luminescent Material

- Platinum (II) octaethylpophyrinketone
- Pro-oxidative actions and photo-oxidation [4]
  - Reduced electron density of porphyrin ring
- High sensitivity to oxygen
  - Applicable in low-oxygen environments
- Ketone increases photostability [5]
- No leaching effects

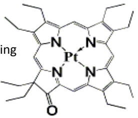
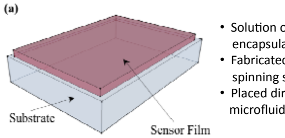


Figure 3. Structure of PtOEPK molecule [6].

Sensor Format



- Solution of indicator and encapsulation medium
- Fabricated by pipetting or spinning solution
- Placed directly below microfluidic device

Figure 4. A single thin film sensor on a generic substrate [5].

Conceptual Diagram

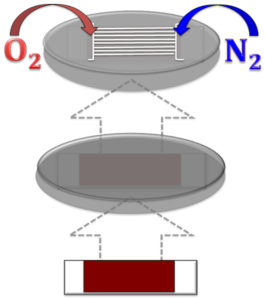


Figure 5. Thin-film oxygen sensor fabricated on a glass slide and placed beneath the microfluidic device for oxygen detection.

Testing and Experimentation

Experimental Procedure

- **Step 1:** Dissolve polystyrene (PS) pellets in toluene
  - 7% w/w solution
- **Step 2:** PtOEPK dye added to PS/toluene encapsulation matrix
  - 1mg PtOEPK: 1mL PS/toluene ratio
- **Step 3:** Transfer PtOEPK matrix into wells of a 96-well plate
  - 32µL per well
  - Allow PtOEPK matrix to gel and dry overnight
- **Step 4:** Test with fluorescent microscopy at varying oxygen gradients
  - Nitrogen saturated (0% O_2)
  - Atmospheric conditions (21% O_2)
  - Oxygen saturated (100% O_2)

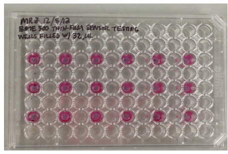


Figure 6. 96-well plate filled with PtOEPK/PS used for experimentation viewed overhead.

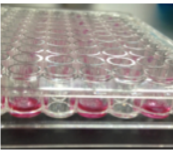


Figure 7. 96-well plate filled with PtOEPK/PS used for experimentation viewed from the side.

Imaging Procedure

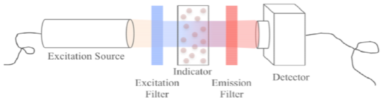
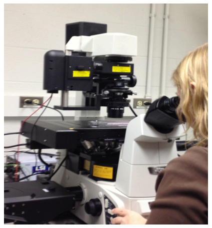


Figure 8. Simplified fluorescent imaging system[5].



- Fluorescence imaging system
- ↑ Intensity for ↓ Oxygen
- Confocal microscope
- Laser used to excite indicator
  - 405 nm
  - 561 nm
- Photomultiplier tube (PMT) used to detect emission
  - •690/60 filter

Figure 9 (left). Imaging of thin-film sensors using multi-purpose three channel confocal located at LOCI.

Results

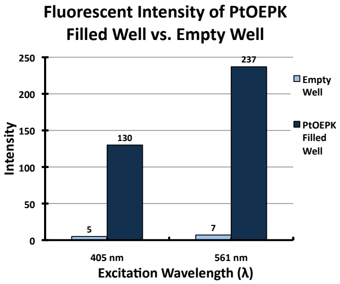


Figure 10. Graph of fluorescent intensity of 96-well plate wells filled with PtOEPK encapsulated in polystyrene compared to empty wells at varying excitation wavelengths.

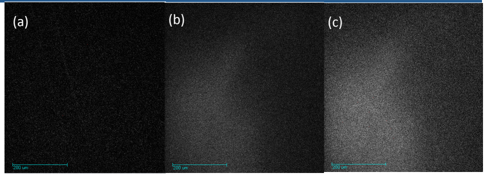


Figure 11. Fluorescent intensity of wells on a 96-well plate. (a) Empty well place excited with a 561 nm laser. (b) A well filled with PtOEPK encapsulated in polystyrene excited with a 405 nm laser. (c) A well filled with PtOEPK encapsulated in polystyrene excited with a 561 nm laser.

Material Properties
- PtOEPK is strong catalyst and makes encapsulation matrix very viscous

Imaging
- λ=561nm optimal excitation wavelength
- Long pass filters are needed to view full emission spectra
- PtOEPK can be tested using fluorescence intensity or luminescence lifetime
- PtOEPK successfully detects oxygen in atmospheric conditions (~21% Oxygen)

Materials and Expenses

Table 1. Materials used for the design project and their corresponding prices.

Material	PtOEPK	Polystyrene	Toluene (anhydrous, 99.8%)
Company	Frontier Scientific, Inc.	Sigma Aldrich	Sigma Aldrich
Catalog No.	040969	182427	244511
Formula	$C_{36}H_{44}N_4OPt$	$[CH_2CH(C_6H_5)]_{11}$	$C_6H_5CH_3$
Mass	743.85 g/mol	1.047 g/ml	0.865 g/ml
Options/Sizes	10 mg	25 G	100 ml
Price	$235.00	$34.10	$28.80
Total cost	**$297.90**		

Future Work

Equipment and Materials

- Long pass filter: 720/60 nm
- Fluorescence imaging system in Ogle lab
- Hypoxia chamber in Ogle lab to create accurate oxygen concentrations
- High grade polystyrene with polymerization inhibitors

Optimization

- Tests to determine optimal thin-film sensors
- Change variables during fabrication:
  - •Percent of PS/toluene encapsulation matrix
  - •Ratio to PtOEPK
  - •Thickness of strips

Standardized Curve

- Collection of data for varied oxygen concentrations
  - •1% to 100% for general trend
  - •1% to 21% for hypoxic conditions
- Intensity data at each oxygen concentration
  - •Increase in intensity with less oxygen
- Stern-Volmer plots [7]
  - •$I_0/I = 1 + K_{SV} [O_2]$

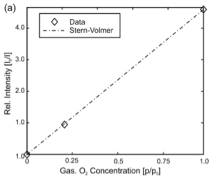


Figure 12. Three-point Stern-Volmer calibration curve for detection of gaseous oxygen [8].

Integration with Microfluidic Device

- Fabrication of thin-film sensors directly onto glass slides
- Placement of microfluidic device on sensor
- Passive pump system with cell media
- Testing cell fusion under hypoxic conditions

References

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