Generation of an accessible and versatile micro-hypoxia chamber

Mid-Semester report

Biomedical Engineering Design 301
University of Wisconsin-Madison
March 6, 2013

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Abstract

Heart attacks have remained the number one cause of death in the United States for a number of years. In order to improve the long-term health of individuals that have suffered heart attacks, and subsequently, damaged cardiac tissue, a new experimental treatment has been proposed: the fusion of stem cells with damaged cardiomyocytes in order to promote healthy growth of new cardiomyocytes to return cardiac function. The damaged tissue is the result of ischemia, and to study the fusion of stem cells with the cardiomyocytes, the experimental environment should be hypoxic. In order to both study the fusion of the stem cells at a small scale, and to monitor the hypoxic conditions of the experiment, a microfluidic hypoxia chamber has been proposed. A PDMS microfluidic device has been fabricated to culture the cells and develop the gas gradient, but a method for real-time monitoring of the oxygen concentration is required. Our team has considered a number of different types of oxygen sensors and selected a platinum-based porphyrin (PtOEPK) as an optimal candidate. We aim to test the effectiveness of the PtOEPK sensor for the remainder of the semester and implement it with the previously designed microfluidic device.

Background

Hypoxia in Cardiovascular Physiology

Despite being on a downward trend since the mid-20th century, heart disease is still the number one cause of death in the United States. All major cardiovascular diseases contributed to 599,413 deaths in the United States in 2009, 24.6 percent of all deaths that year[1].

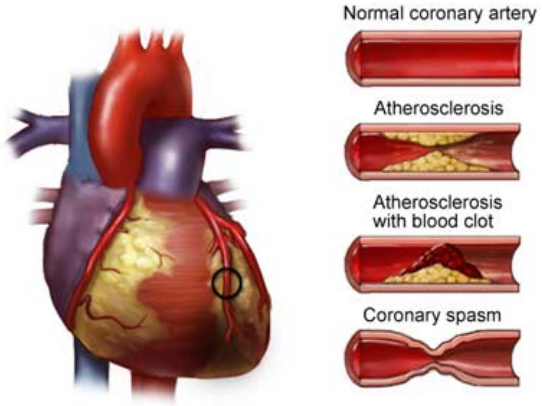

Figure 1: A number of different causes of cardiac ischemia[1]

One of the severe symptoms of heart disease is heart attacks, or myocardial infarctions. A variety of factors can lead to heart attacks, but the primary cause is insufficient blood supply to the heart, or cardiac ischemia, leading to the death or damage of sections of cardiac muscle tissue. The damage occurs because the lack of oxygen, or hypoxia, consequents in cell apoptosis, or cell death. Immediate treatment of the patient often includes cardiopulmonary resuscitation (CPR) or electrical shock (defibrillation). These treatments are used to prevent fatalities after heart attacks, but do not address the long-term effects of heart attacks[2].

Current methods of long-term treatment include cardiac rehabilitation and lifestyle change in order to improve the function of the failing heart sections, but new experimental methods are being investigated in order to provide a more permanent and effective solution. One such avenue of research is examining the use of stem cell therapy in repairing the damaged cardiac tissue. The hypothesis is that transplanted stem cells will fuse to damaged cardiac tissue and promote growth of new, healthy cardiomyocytes. Similar research using progenitor cells (similar to stem cells) shows the positive rehabilitative effects and potential future in stem cell therapy in treating patients[3]. In order to study potential use of stem cells in damaged heart tissue, stem cell and cardiomyocytes must be cultured in a hypoxic environment similar to that of the heart, post-myocardial infarctions.

Microfluidics in Hypoxia Research

Two of the primary limitations of basic life science research are cost and resources. Experiments involving cells and other biological materials are expensive and utilize a lot of resources to maintain or conduct experiments. In order to minimize both, the use of microfluidic devices has become popular within the last decade, with an increase of 38 to 1270 published uses of such devices from 2000 to 2010.

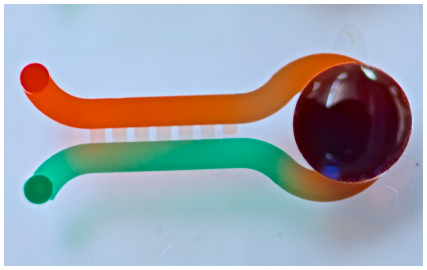

Figure 2: A microfluidic device

Microfluidic devices manipulate minute amounts of liquids and cells in order to study cellular and biological processes at a micro-level. The design and implementation of the device itself greatly varies between research applications because of the specificity of the research that they are used in, but they often share some of the same fundamentals. More than 45% of microfluidic devices published in 2009 used polydimethylsiloxane (PDMS) as the primary material in their composition[4]. PDMS is a particularly desirable material because it is biocompatible, hydrophobic, easily manipulated, and gas permeable.

A PDMS microfluidic device would be an appropriate candidate for mimicking hypoxic environments. The primary characteristic that makes it an ideal candidate is its ability to develop a hypoxic gradient. The ability to introduce gasses on either end of the device allows a gradient to develop through the gas permeable PDMS. In addition, the micro-nature of the device allows the control of oxygen concentration to be more finely tuned and lessens the use of critical reagents, gasses, and cells, minimizing cost. The use of small amounts of oxygen demands that the concentration gradient be monitored precisely, accurately, and in real time in order to confirm that the hypoxic environment is being mimicked properly and as desired.

Current Technology

Current methods of monitoring oxygen are primarily concerned with the large-scale sensation of normoxic and hypoxic conditions. Traditionally and commercially available oxygen sensors include solid-state potentiometric equilibrium sensors, limiting current amperometric sensors, semi-conducting metal oxide sensors, and optical sensors[5]. These devices often consume oxygen and are disruptive in cellular research, which make them non-ideal for the desired application. There are also a number of solutions that sense oxygen at a smaller scale, but are generally reserved for individual research purposes. These range from protein sensors to electrical sensors, but there are a few that serve the desired function of monitoring oxygen in a microfluidic device.

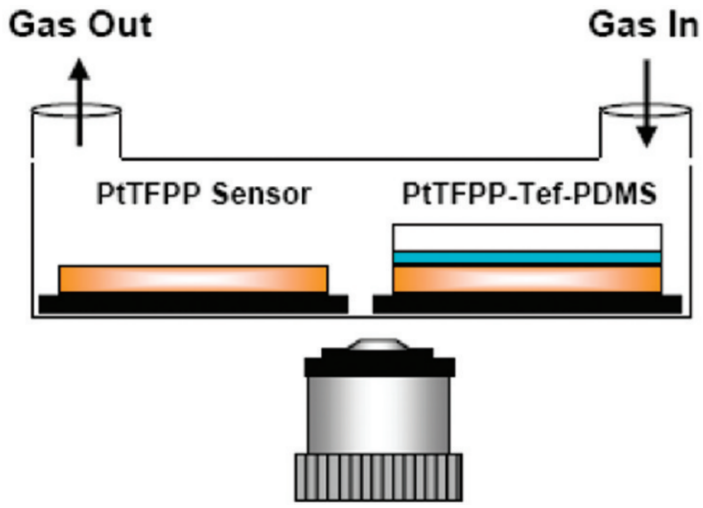

Figure 3: A thin film sensor developed at the University of Maryland

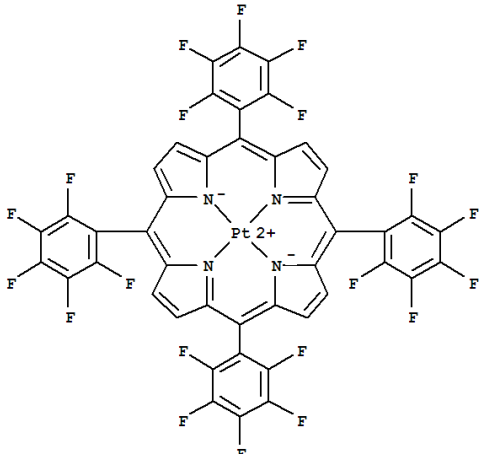

Figure 4: PtTFPP protein

One such oxygen sensor is a non-invasive thin film sensor developed at the University of Maryland. This sensor uses a platinum-based porphyrin sensor (PtTFPP) in a PDMS thin film layer, pictured in Figure 3. Once the sensor is in contact with oxygen, it can be excited by fluorescent light and will emit a given emission light that is directly proportional to the concentration of oxygen within the measured environment[6].

Another similar oxygen sensor is one developed for research at the University of Michigan and the MacDiarmid Institute for Advanced Materials and Nanotechnology at the University of Canterbury. This thin film sensor utilizes a ruthenium tris (2-2' dipyridyl) dichloride hexahydrate (RTDP) oxygen sensitive dye with a PDMS device in order to monitor the oxygen[7].

Both of these devices, among a number of other devices manufactured by research labs globally, function in a manner that satisfies some of the cursory requirements necessary for monitoring oxygen in hypoxic studies of cardiac stem cell therapy, but they fail to address a number of other parameters, including cost, ease of use, and application specificity. These sensors are often tailored to the specific experiments that the research is concerned with and so a more appropriate and custom solution would be more applicable for studying the hypoxic conditions of cardiac stem cell therapy.

Previous Work

In previous semesters, a number of steps have been taken to develop a microfluidic device and sensor for use in studying stem cell fusion with damaged cardiac cells in hypoxic conditions.

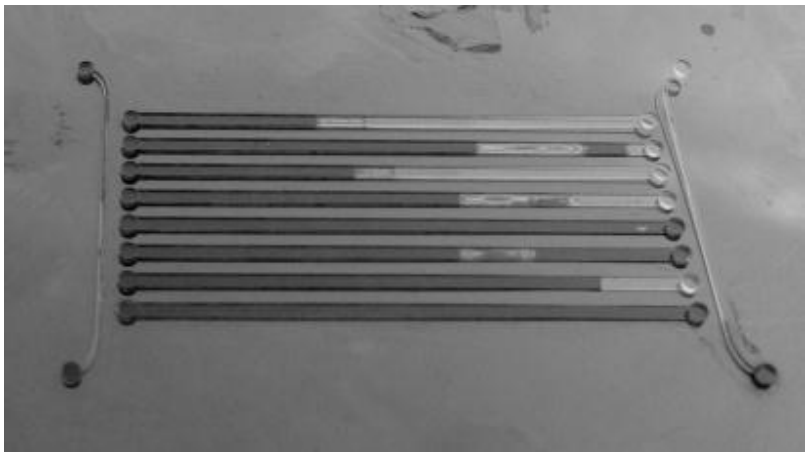

Figure 5: The microfluidic device developed in spring 2012

In the first semester of the project (spring 2012), the design team worked largely on designing and fabricating the microfluidic hypoxia chamber. The master template for the device, as pictured in Figure 5, was successfully designed for repeated construction with PDMS. The device consists of a number of lateral micro-channels where cells will be cultured surrounded by passively pumped media, and two

longitudinal channels where oxygen and nitrogen gas will be passed in order to develop the hypoxia gradient. The entire process for building the device consists of an approximately 45-minute production protocol followed by a four-hour heating process.

In the second semester of the project (fall 2012), the design team worked on the selection and implementation of an oxygen sensor for real-time monitoring of the oxygen concentrations across the device. The sensor needed to be compatible with the previously design microfluidic device, accurately sense the oxygen, and be non-cytotoxic to the cells. The team selected a platinum-based porphyrin sensor (PtOEPK) and began preliminary testing in order to develop a standard curve for the sensor. Their work was not completed due to time constraints and errors in their testing protocol.

Problem Statement

High accuracy oxygen detection has become a critical step in understanding various physiological effects of hypoxia. The purpose of this project is to test, redesign and produce an oxygen sensor that can be used with the microfluidic-based hypoxia chamber. Previous work on this design project has produced a functioning microfluidic-based hypoxia culture device and preliminary work on the oxygen sensor. This semester will focus on testing the hypoxia chamber, re-examining possible oxygen sensors, and implementing the two systems together. After confirming the microfluidic hypoxia culture device and oxygen sensor have been assembled properly, the device will be used in experiments involving oxidative stress, ischemia, and reactive oxygen species (ROS)-mediated cellular pathways.

Design

Design Alternatives

PtOEPK Sensor

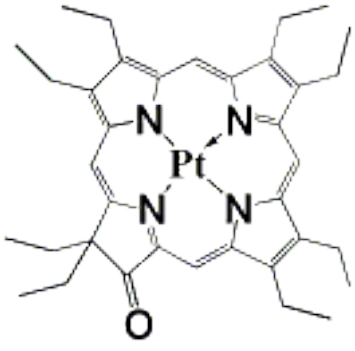

Figure 6: PtOEPK protein

Platinum (II) octaethylporphyrinketone (PtOEPK) is a fluorescent porphyrinmolecule. It is used with a polystyrene matrix as a solid state oxygen detector. It acts as a hydrophobic dye, which has excitation wavelengths of 408 nm and 601 nm and an emission wavelength of 791 nm.

The PtOEPK sensor has a relatively high sensitivity to oxygen with high photo-stability compared to the other methods that were considered. It also introduces a relatively low cost as the thin film sensor could be reused for multiple experiments, over the course of a few months,. However, its biological nature leads to

variability during detection. As it does not provide direct measurement, there might be variation between the actual oxygen concentration and the fluorescence the microscope reads. Moreover, due to its non-standard excitation and emission wavelengths, it is not compatible with the microscopes available in the university. Extra filters would have to be purchased for future work with this sensor, albeit at relatively low cost[8,9].

Bead Injection Spectroscopy (BIS)

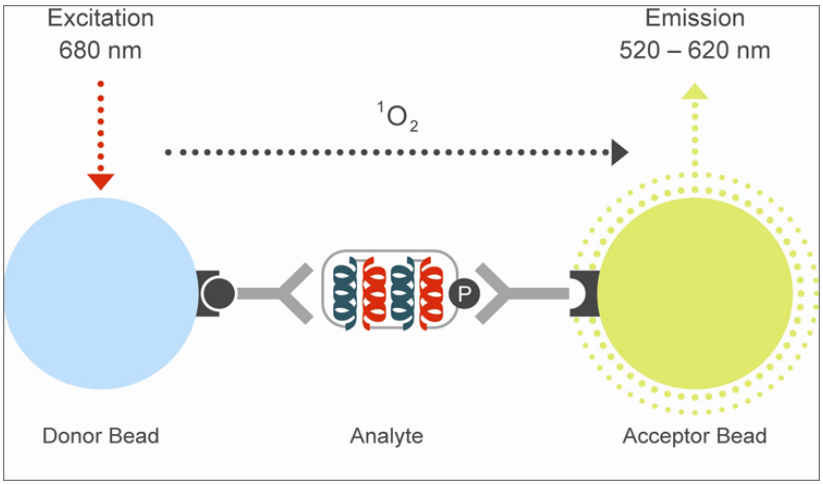

Figure 7: a typical bead injection system

Bead injection is a recently developed technique where a specific volume of bead suspension is injected and acts a sensing layer, which is disposable. As an oxygen sensor, the beads would be bonded non-covalently to oxygen phosphorescent probes. The beads are then trapped in cells and placed under a non-destructive spectroscopic detector. The fluorescent signals from the beads would then be picked up and measured.

In particular, the acid form of Pt-tetra (paracarboxy-phenyl) porphyrin tetraethyl ester, Pt-(p-COOH)4PP was used as the oxygen-dependent quenching phosphorescence here. The excitation wavelength used was 509 nm and emission wavelength was 550 nm.

With the BIS method, the beads can be placed proximal to the cells and measurements can be made at the spot. It also allows a time scale of minutes for oxygen detection. Moreover, it avoids the problem of sensor surface degradation that the other optodes have. However, the biggest drawback of the BIS method would be the consumption of oxygen during detection, which would not be desirable as this can significantly skew the accuracy of the oxygen concentration measurement. Additionally, there is only a limited amount of literature available on the success of this method, so its accuracy and viability has not been verified[10,11].

Electrical Sensor

The electrical sensor makes use of the energy generated during the collision of oxygen molecules and a fluorescent chemical in its excited state. An optical fiber

carries a light at around a 475 nm wavelength to a probe. There is a layer of hydrophobic sol-gel at the tip of the probe, which is excited by the light from the fiber and emits energy at around 600 nm. The presence of oxygen molecules generates excess energy, which is then collected by the probe and sent to the spectrometer.

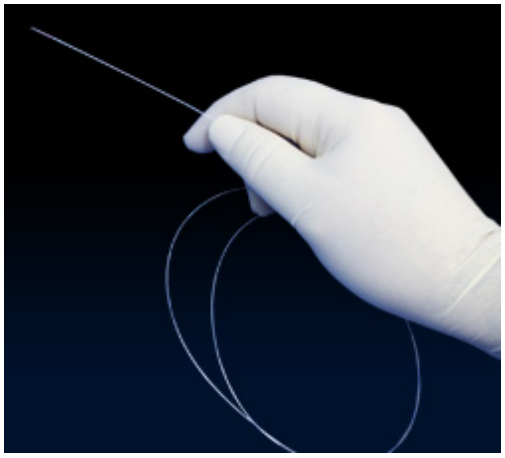

Figure 8: a typical optical fiber sensor

This method is relatively accurate compared to the other sensors due to its strong basis in fiber optics. It also produces discrete quantitative data, unlike the other sensors which rely on color detection. Moreover, it is reusable for multiple experiments. On the other hand, it requires an array of individual probes to provide a complete curve of data, and would therefore be a large initial investment. Also, the hypoxia chamber designed by previous teams would have to be scrapped in favor of a completely new design as it was created for use with a protein sensor, limiting the relevance that these electrical sensors have to this particular project[12,13].

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	5
Cost (15%)	3	3	2
Total	72	56	64

Table 1

Observing Table 1, sensitivity and accuracy are the two most heavily weighted categories. Accuracy is a measure of how close a measurement is to the true value. Sensitivity (more commonly understood as its equivalent: precision) is a measure of how closely grouped a number of measurements are to a given value that may not necessarily be the true value. Something that is both accurate and sensitive will not only give a measurement close to the true value, but will also remain close to that value over a number of measurements. An oxygen sensor necessarily must be able to satisfy both of these functions. The electrical fiber optic sensor scored best overall for the two categories combined because according to the supplier, it provides consistent measurements. Errors in sensor systems generally appear when one signal has to be translated into another type of signal. While electronic sensors require only one signal conversion, both BIS and PtOEPK sensors require at least two conversion steps. Protein sensors are subject to degradation, mishandling, or improper procedure when making them, and hence may lose their accuracy. Bead injection Spectroscopy (BIS) consumes oxygen during operation, earning it the lowest score for sensitivity and accuracy because this consumption could significantly skew the measurements. In addition, bead injection spectroscopy (BIS) is currently under development and there is disagreement concerning its actual sensitivity and accuracy.

Integration is a “measurement” of the ability of the sensor to be integrated with the current channel design. Our design must be compatible with the cell culture microfluidic device that creates the hypoxia condition. The PtOEPK sensor scored highest in this category because it can be fabricated into a thin sheet which can be easily attached onto the top of the culture device. Utilizing BIS or an electrical sensor would require modification of the channel and/or cell culture protocol to align with the sensor’s method of oxygen measurement.

Ease of fabrication is a measure of how simple the device would be for the design team, or a lab associate, to create. This is a relatively important standard to consider because as a research tool, new sensors will be made frequently when needed in experiments. The electrical sensor scored the lowest in this category because these fiber optic devices involve complicated and sensitive fabrication methods that would be difficult for the design team to reproduce. A system of the electronic fiber sensors could be purchased, but this would be outside of budget. BIS scored the highest in this category because the liquid sensor is available for use as it comes, only requiring simple dilution, and does not require an extra physical sensor matrix as is necessary for PtOEPK. However, PtOEPK is still quite manageable, just time-consuming.

Response time is a measure of how quickly the sensor adjusts to and reports changes in oxygen concentration. The biological sensors will report changes in oxygen concentration by exhibiting a color change, which is relatively fast, but is on the order of seconds to minutes. Depending on the sensor purchased, the electronic system that will be used to analyze the signal, have fast response times on the order of milliseconds. Another factor that can affect response time is the calibration of the

sensor and the software and analysis tools. These tools have relatively small impact on the time for response, but can contribute to errors[15].

Cost is important to the project. None of the options are especially cheap, but the electrical sensors border on $600 per sensor. Our applications would require an array of sensors, multiplying the cost. A 10mg vial of PtOEPK costs around $235 and BIS of same quantity would cost $250 ~$300. Although the electronic sensor can be easily used for up to a year long, the PtOEPK sensor protein can also last more than three months, which made it possible[16,17].

As can be seen in Table 1, PtOEPK scored the highest by a fair margin, justifying the use of PtOEPK as the preferred option for sensor fabrication. The final design section further describes how this protein sensor will be implemented and made compatible with the current channel design.

Final design

General information & specification

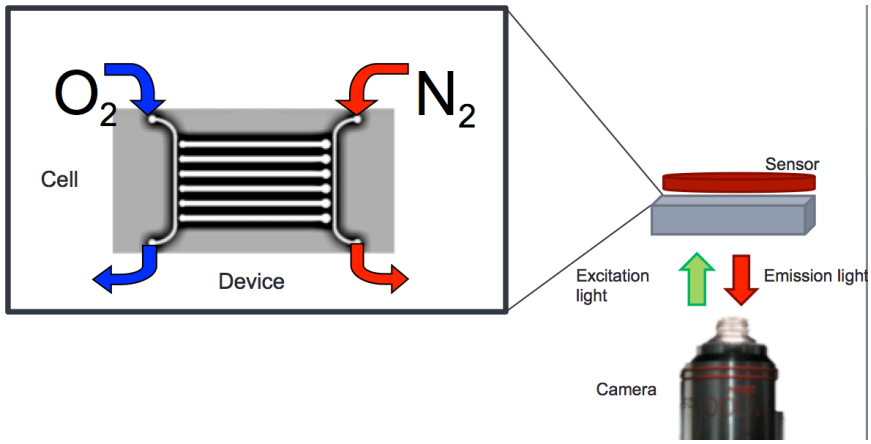

Figure 9: Final design operational theory

Our final design utilizes a similar method to the previous semester, but with some notable changes. PtOEPK will still be used as the protein sensor, but the 96-well plate used last semester to house the protein will be abolished in favor a single "sheet" of protein material. The 96-well plate was found by the design team to be difficult to focus during imaging, and the small wells caused a meniscus effect in the settling of the protein solution (see Figure 10). Both of these factors are detrimental to the imaging of the protein, which is required to obtain an accurate oxygen concentration measurement. Instead, the protein solution will be formed into a thin film utilizing two glass slides, plastic overlays, and capillary action. Two slides will be placed approximately 1 millimeter apart, and the protein solution will be injected between them and allowed to spread throughout the space between the slides by capillary action. It is important to note that this design is preliminary and subject to change if,

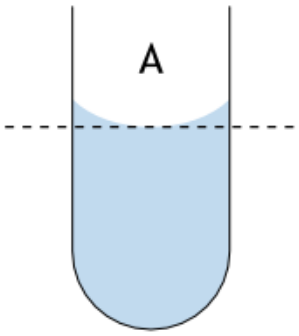

Figure 10: Meniscus effect

through testing, it is determined to be inadequate for our requirements.

Future work:

Aim 1: Design and develop a sensor matrix that can be integrated onto the device.

The sensor matrix is the framework of the sensor that provides physical integrity and structure. To avoid the unnecessary usage of the expensive PtOEPK fluorescent protein, we will first optimize the sensor matrix without the protein in order to maximize the effectiveness of the sensor. This will also allow later sensitivity testing to be performed in conditions similar to those of real experiments, thereby increasing the reliability of the results. The sensor matrix will be fabricated using polystyrene dissolved in toluene to form a thin layer (see Appendix C). Specifically, the solution of polystyrene in toluene will be loaded between two glass slides underneath a plastic overlay. After the solution has hardened, the sensor matrix will be cut to desired dimensions and integrated onto the device in a manner yet to be determined.

Aim 2: Testing of the PtOEPK sensor and determination of the calibration curve

After the design is optimized, the PtOEPK sensor will be added to the matrix for sensitivity testing. Several different tests will be performed on the device . To achieve the best model of actual experimental conditions, the sensor will be integrated onto the cell culture device during the testing. For the first test, the sensor and the device will be placed on a cell culture dish and then sealed into the oxygen chamber, which controls the concentrations of oxygen. Oxygen concentration will be manipulated and intensity of emission light will be recorded accordingly. The device, with and without media, will be tested to examine the effect of cell culture media on sensor function. A calibration curve of emission intensity versus oxygen concentration will be plotted. Finally, cells will be seeded into the device and the sensor system will be tested in actual experimental conditions.

An important point to address here is that the design process will be required in multiple steps. We have not yet determined how to perform many of these tests and new solutions will likely have to be implemented. For example, whether the device and the sensor should be permanently attached at the beginning of each experiment or attached to the device shortly before remains to be decided. Also, the exact dimensions of the sensor matrix and the concentration of PtOEPK sensor will need to be optimized according to test results.

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Figure References

[1]<http://www.riversideonline.com/source/images/image_popup/ans7_myocardialischemia.jpg> *Retrieved March 3, 2013*

[2] Picture taken by Jason Yu on his squid channel

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[5] Picture taken by Jason Yu of master template from last semester

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Appendix - A: Product Design Specifications

Development Oxygen Sensor for Microfluidic Hypoxia Chamber Product Design Specifications February 6, 2013

Client: *Professor Brenda Ogle, PhD*

Advisor: *Professor John Puccinelli*

Team: *Sarvesh Periyasamy, Roland Pomfret, Lok Wong, Jiaquan Yu,*

Function:

High accuracy oxygen detection has become a critical step in understanding various physiological effects of hypoxia. The purpose of this project is to test, redesign and produce an oxygen sensor that can be used with the microfluidic-based hypoxia chamber. Previous work on this design project has produced a functioning microfluidic-based hypoxia chamber and preliminary work on the oxygen sensor. This semester will focus on testing the hypoxia chamber, re-examining possible oxygen sensors, and implementing the two systems together. After confirming the hypoxia chamber and oxygen sensor have been assembled properly, the device will be used in experiments involving oxidative stress, ischemia, and reactive oxygen species (ROS)-mediated cellular pathways.

Client Requirements:

- Test the reliability and accuracy of the current preliminary oxygen sensing design using a 48 well plate.
- Improve the system so that it can detect the oxygen concentration from 100% oxygen to 1% oxygen with an accuracy of at least 2%.
- All components of the device must be compatible with the microfluidic device (made with PDMS).
- The device must not have any cytotoxic effects on the cells being cultured, which might be in close contact with the device.
- The sensor may be placed on the top or bottom of the microfluidic device without affecting microscopy.
- The protocol should be easily repeatable and the fabrication should not be time consuming

as new sensors have to be made for each experiment.

Design Requirements:

1. Physical and Operational Characteristics

a. ***Safety:*** The oxygen sensor must be safe for individuals to handle in a laboratory setting. The device must be sterile in accordance with the cell culture protocols that the device will be used with. The materials used should not leak any toxic residue in high temperature situations (such as when autoclaved).

b. ***Accuracy and Reliability:*** The accuracy and reliability of the sensor will depend greatly on the fluorescent dye and microscope. We are aiming for a less than 2% error rate within each sample and less than 5% variation between samples.

c. ***Life in Service:*** The device and the oxygen sensor for it are of disposable nature. After each experiment, which can last up to approximately seven days, both the device and the sensor will be disposed of and new ones will be made for the next experiment.

d. ***Shelf Life:*** Since a device is made as needed, shelf life is not a significant factor in this project. We propose a one month functional storage time.

e. ***Operating Environment:*** The oxygen sensor should be stable in the incubator (37 C 5% C02). The sensor will be in close contact with and should not be affected by cell culture media, serum, and other chemicals. The sensor should also give a reliable fluorescent signal in a room temperature environment for an hour in order to provide enough time for microscopy.

f. ***Ergonomics:*** The device should be easy to use by a variety of researchers. It should be small enough to hold in the hand and be simple enough to be operated by inexperienced users.

g. ***Size:*** The dimension of the sensor should be made according to the dimension of the device so that the sensor can be assembled to the device. The device has a six-inch diameter**.**

h. ***Weight:*** Weight is not a critical design constraint at this time. The weight of the device should allow it to be carried easily in the hands of the researcher. We aim to keep the mass under 50 grams.

i. ***Materials:*** The materials used should be standard materials used for solid state sensors. Possible materials include fluorescent dye, PDMS, polystyrene and other standard cell culture materials.

j. ***Aesthetics,*** *Appearance and Finish:* Since the sensor may be placed over the microfluidic device, the sensor area above the culture should be transparent and clear so that microscopy is not affected. The sensor should have sufficient sealing with the microfluidic devices.

2. Production Characteristics

a. ***Quantity:*** There will be one oxygen-detecting unit produced for one micro-hypoxia chamber.

b. ***Target Product Cost:*** The proposed budget for this semester is $500**.**

3. Miscellaneous

a. ***Standards and Specifications:*** The product is not drug related and does not require any FDA approval. Neither human nor animal testing is required so there are no concerns for approval. The only protocol that the device must adhere to is the mammalian cell standard operation procedures and specifications.

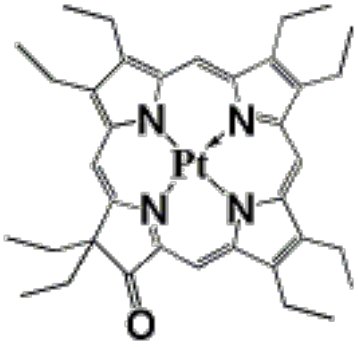
b. ***Customer:*** The device is a custom design for Dr. Brenda Ogle and graduate student Brian Freeman. Eventually, other members of the Ogle lab will be utilizing the device and, if applicable, other labs doing similar research at other institutions.

c. ***Patient-related Concerns:*** The device will be used in a purely research setting so there are minimal concerns regarding patients. There is no patient data or other sensitive information at risk.

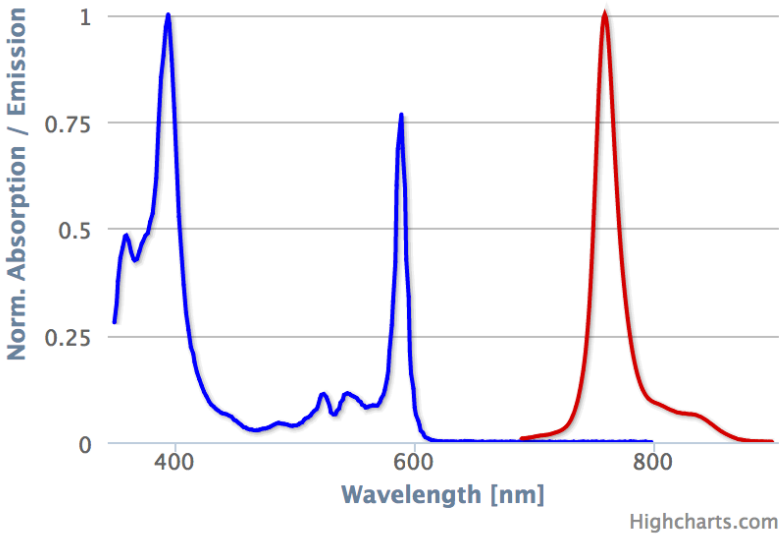
d. ***Competition:*** Most of the competing devices are from various other labs and universities and are therefore optimized for their own research projects. The University of Michigan created a well known thin-sensor film which is too expensive for the Ogle Lab. The Microtechnology Medicine Biology Lab at the University of Wisconsin-Madison also created an oxygen sensing system which is not optimized for Dr. Ogle's purposes.

Appendix - B: PtOEPK sensor datasheet

Basic Information



Common name(s):PtOEPK
IUPAC name:platinum(II) octaethylporphyrinketone
Chemical Formula:C36H44N4OPt
Molecular Weight:743.30g / Mol
Substance Class:Metal-Ligand Complex
Spectra:



Overview

Absorption λ_{max}

398 nm

Emission λ_{max}

760 nm

Solvent

chloroform

Molar Abs. Coefficient

-

Quantum Yield:12%
Method: Reference Standard
Solvent: chloroform
Solvent: toluene

Appendix - C: Preliminary Testing: Hypoxia Chamber Project

Feb 21, 2013

Purpose: To prepare a thin film sensor using a polystyrene matrix and a PtOEPK sensor

Testing protocol:

1. Prepare PtOEPK matrix at different volume ratios of matrix: PtOEPK
    a. Dissolve PS pellets in toluene solution (10% w/w)
    b. PtOEPK dye added to matrix (10 mg into 1mL)
    c. Transfer 32 µL of PtOEPK matrix into each well of the 96-well plate
    d. Covered with aluminum foil.
2. Allow the PtOEPK matrix to stand in wells for 24hrs at room temperature in dark to dry