DEVELOPING AN OXGYEN DETECTION METHOD FOR A MICROFLUIDIC-BASED HYPOXIA CHAMBER

Final Report

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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 regrown. A proposed treatment for reversing this damage is the fusion of stem cells into the damaged tissue. Research has 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 a previous design team. The oxygen detection 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 sensors consist of platinum (II) octaethylporphyrinketone (PtOEPK) in a polystyrene encapsulation matrix. The sensors were fabricated and tested in 96-well plate and imaged for fluorescent intensity using a confocal microscope. Intensity levels at atmospheric conditions (approx. 21% O_2) were measured and recorded. After testing, the device demonstrated sensitivity to oxygen and provides a viable option for oxygen detection and monitoring within a microfluidic construct.

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BACKGROUND

Heart Attacks and Hypoxia

Each year, 600,000 Americans have heart attacks, which are resultantly the leading killer of both men and women in the country. They are responsible for 25% of all deaths in the United States [1]. A heart attack is commonly brought on by coronary heart disease, which causes blockages in the heart that restrict the flow of blood to the vital muscle [2]. When a patient has a heart attack, his or her cardiac cells experience a lack of oxygen due to the restricted blood supply to the heart. This phenomenon is called ischemia. As the cells are deprived of oxygen and experience ischemic injury, they go through cell apoptosis, or cell death. This results in significant damage to the heart tissue, which is detrimental to the health of the patient. Such damage necessitates an effective treatment to restore the tissue back to health. Since this occurs in vivo, or inside the body, it is difficult to study prospective treatments. A newly proposed treatment involves the transplantation of stem cells into the damaged cardiac cells. If the treatment is successful, the stem cells will fuse to the tissue and will begin to produce new, healthy cardiac cells. Studies have shown that this cell fusion is more likely to occur under hypoxic conditions, where the cells are in environments with low oxygen concentrations. Such conditions can be mimicked with regulated environments called hypoxia chambers.

Microfluidic Devices

Microfluidic devices are devices that perform small-scale experiments with fluids in small channels [3]. Cells are commonly incorporated into such devices, and one example of a microfluidic device is a hypoxia chamber. Hypoxia chambers create low levels of oxygen by making a gradient as the gas diffuses through the device. Since extremely low levels of oxygen are used, an accurate and effective method for detecting the exact oxygen concentration is necessary to make this proposed treatment successful. In order to ensure that cells are truly under hypoxic conditions, the oxygen concentration must be accurately determined.

Past Semester's Work

In the spring of 2012, Professor Brenda Ogle and her lab's team were the clients of a BME 301 design team. The team was tasked with producing a microfluidic-based hypoxia chamber to test the proposed treatment of stem cell fusion into damaged cardiac cells. The team successfully designed and manufactured a master template to create a microfluidic device for Dr. Ogle's work on stem cell fusion under hypoxic conditions. Their device is show in **Figure 1** to the right. The devices are produced in approximately 45 minutes, followed by a four-hour heating process [3]. During experiments and testing with the device, nitrogen and oxygen gases are passed through the channels to create the oxygen gradient. Ultimately, a passive pump system will be used to pass cell media through the channels.

Figure 1. Master slide of microfluidic device developed by BME 301 [3].

PROBLEM STATEMENT

Understanding the impact of hypoxic stress on the behavior of cells transplanted into the heart following ischemic injury can be achieved by mimicking facets of the in vivo environment in an in vitro system. Control of oxygen levels in cell culture has traditionally been achieved using large hypoxia chambers at one concentration at a time. Consequently, microfluidic devices have been proposed to improve accessibility, versatility, and to generate overall function of hypoxic environments. The goal of this project is to design and produce a microfluidic-based hypoxia chamber to facilitate experimental investigations involving oxidative stress, ischemia, and reactive oxygen species (ROS)-mediated cellular pathways. Previous semester's work on this design project produced a functioning microfluidic-based hypoxia chamber. This semester's work will focus on developing a means to accurately monitor and detect the varying oxygen concentrations and gradients present in the chamber.

CURRENT DEVICES

Microfluidics is a relatively new field, with newfound biological applications. Consequently, there are no current devices readily available for oxygen detection in microfluidic devices. Currently, there are larger scale commercial devices available for oxygen detection and research devices created for oxygen detection applications for that specific lab.

Commercial Devices

Commercial devices include large thin-film sensors and electrodes. The thin film sensors available are extremely high in cost and offer a limited variety in luminescent material that can be used for detection. These qualities make these sensors unattractive for our design purposes. The oxygen sensing electrodes need to consume oxygen to perform a redox reaction to detect the gradient present, as shown in **Figure 2**. This not only affects the environment the electrodes are placed in, but it also causes very poor accuracy [4].

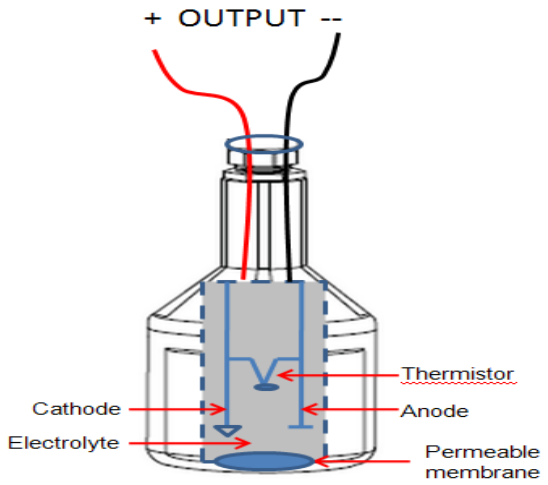


Figure 2. DO6400 Series Dissolved Oxygen Sensor with NI Wireless Sensor Networks (WSN) provided by National Instruments [4].

Research Devices

The research devices available offer a great deal of insight on how to best develop an oxygen-sensing device for microfluidics. The University of Michigan and the MacDiarmid Institute for Advanced Materials and Nanotechnology at the University of Canterbury have created thin-sensor films for oxygen detection in microfluidic devices, as illustrated in **Figure 3**.

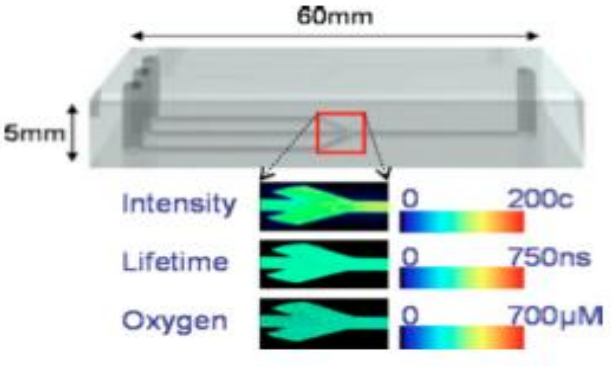


Figure 3. Illustration of fluorescence intensity and lifetime imaging in microfluidic devices using the method developed at the University of Michigan [5].

These devices do not offer the ease of use and affordability desired. Additionally, there exist other thin-film sensor, microparticle/nanoparticle sensors, and water-soluble/macromolecule probes that have been manufactured by research laboratories using a variety of luminescent material. Additionally, these research labs have determined that two methods for oxygen detection are possible – measurement of intensity or measurement of lifetime [5]. In intensity detection, the fluorescence intensity of the indicator material is proportional to the concentration of oxygen. In lifetime detection, the exponential decay rate of the indicator materials is compared to its known decay rate and lifetime.

DESIGN REQUIREMENTS

For the device to be functional at a high accuracy level and work specifically to the client's needs, several important design specifications must be met. First, the oxygen sensing device must be able to detect oxygen concentrations from 1 – 21% O_2 with an accuracy of +/- 2 to 3%. The device must maintain and repeat this high accuracy through frequent use, as many experiments will be carried out on a daily basis. The life in service of the device needs to be approximately two weeks, or lasting the length one experiment in the Ogle Lab. Also, to properly function with cells and cell culturing, the device needs to operate in an environment of 37°C and 5% CO_2. To support imaging of the cells, the device must operate and not be affected by fluorescent exposure. Overall, the device must have high ease of use and ease of assembly while maintaining a low cost.

DESIGN ALTERNATIVES

Oxygen Sensor Formats

Thin-film Sensors

Thin-film type sensors have already been successfully in laboratory microfluidic devices [6][7][8], including work with cell cultures, and are generally fabricated by either pipetting or spinning solutions of the indicator and encapsulation medium onto a substrate of interest such as a glass slide [9][10]. A generic thin film-sensor is shown in **Figure 4.** There are many different ways to create such thin-film sensors. First, the indicator solution can be pipetted directly into laser-treated ridges of the glass slide and place the microfluidic device on top of the glass slide, with the indicator side face-up. Second, the thin-film sensor may be created onto a glass slide, and the microfluidic device may be placed directly on top of the oxygen-detecting material. Lastly, fabricated thin layers have also been lithographically patterned using PDMS stamps as shown in **Figure 5** on the following page.

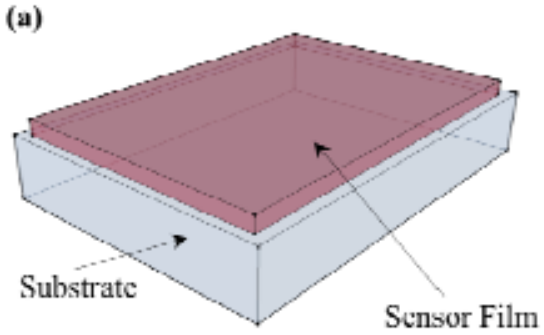


Figure 4. A single thin-film sensor on a generic substrate [11].

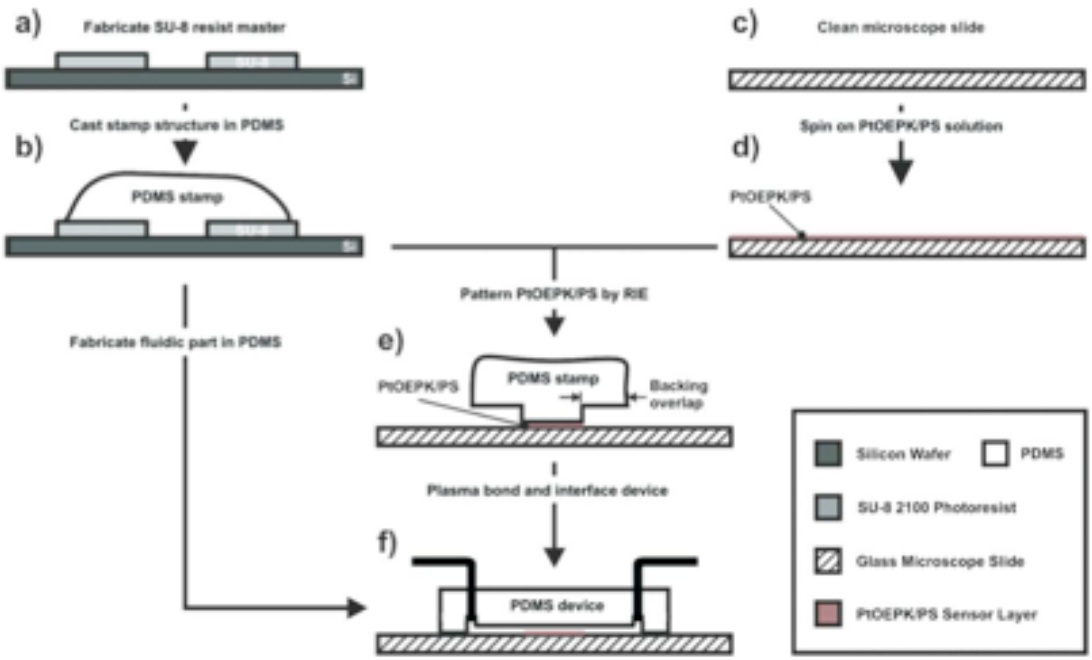


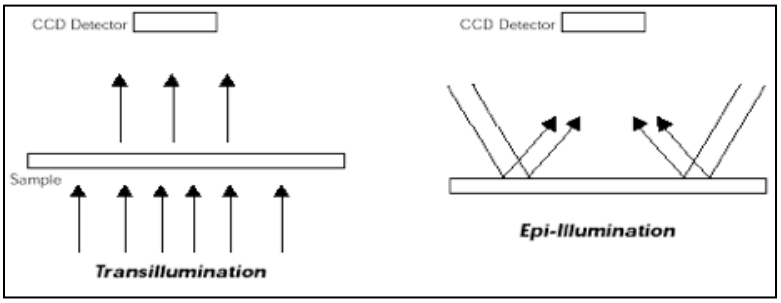
Figure 5. Schematic of the sensor fabrication process showing stamp fabrication in PDMS. [6]

For the above fabrication process, the silicon wafer master slide is used to shape the PDMS stamp of the device. Then, the indicator, in this case PtOEPK with a polystyrene encapsulation matrix, is pipetted onto a glass slide. The PDMS stamp is then used to shape the sensor for the microfluidic device before the PDMS microfluidic device is bonded to the glass slide.

Soft-lithography and plasma etching with reactive ions can be used to fabricate a polymer microfluidic cell-culture device with an integrated optical oxygen sensor. The indicator can be spin-coated to form sensor films of variable thickness, depending on the spin speed of the indicator solution [12]. In addition, encapsulating the sensor in polymer or sol-gel matrix will reduce the likelihood of unwanted interactions.

Microfluidic devices made of PDMS are transparent, allowing sensor dye to be excited by either process of trans-illumination or epi-illumination. The difference between these two processes is shown in **Figure 6**.

Figure 6. Diagram of the different illumination processes [6].



In **Figure 6**, the sample would be the channels of cells and the thin film indicator would be our CDD detector.

Illumination involves transmitting an energy source, such as a LED (which we

will most likely use because they are inexpensive, may be pulsed or modulated, and are commonly used as excitation sources for oxygen sensors elsewhere) through the sample, while epi-illumination involves transmitting an energy source at an angle. Both will excite fluorescence energy causing a visible emission that can be measured.

Microparticle/Nanoparticle Sensors

The desire to create a versatile sensor platform with both the advantages of indicator encapsulation and the possibility of intracellular measurements led to the development of microparticle/nanoparticle sensors. Optimal spectral characteristics and sensitivity to oxygen, excellent photostability, low cytotoxicity and phototoxicity, are loaded into cells by simple transfection procedures and subsequently analyzed by high-resolution fluorescence microscope. In addition, this sensor technique has been widely used for studies with cellular hypoxia in microfluidic devices.

Microparticle and nanoparticle oxygen sensors have been fabricated by doping polymer or silica beads with luminescent indiciator dye [13] or by grinding indicator-doped ormosil. These microparticle and nanoparticle sensors have been used directly [14] or embedded in another material such as silicone [13] or hydrogel to form thin-film sensors [15]. This production process is shown in **Figure 7**. Microparticle and nanoparticle sensors could be integrated in the cell culture area by adding the particles to silicone within the channels of the microfluidic device.

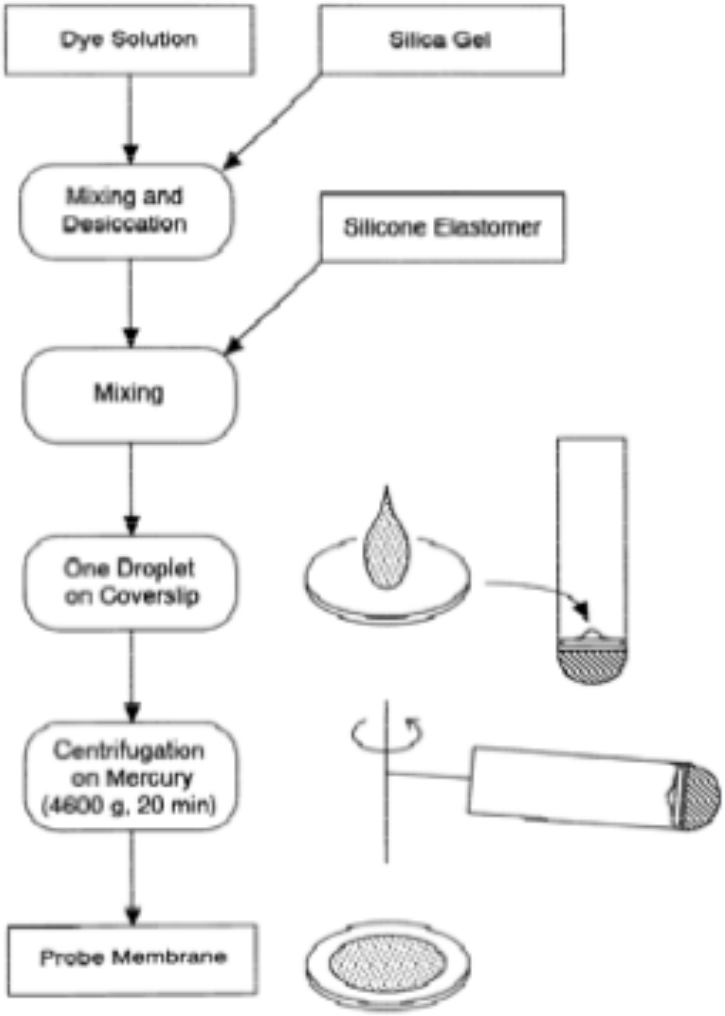


Figure 7. Preparation of oxygen-sensitive probe membrane. This procedure yields a circular membrane that is composed of a thin layer of dyed silica gel beads and a silicon rubber film, supported by a microscope glass cover slip [13].

Silicone rubber is usually used to coat dyed beads because of its higher gas permeability compared to other possible polymers used. It is also non-toxic and transparent for using a LED source to excite the sensor dye. In addition to silicone rubber being toxic, it won't react with the cells. So encapsulating the sensor in silicone, some other polymer or sol-gel matrix will reduce the likelihood of unwanted reactions.

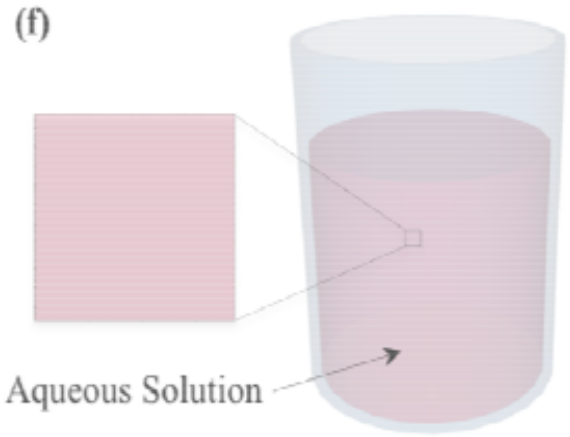


Figure 8. Diagram of water-soluble sensor compound dissolved in aqueous media [11].

Water-Soluble/Macromolecular Probes

The final general sensor platform is the dissolved, macromolecular probes. This format uses water-soluble probes to detect oxygen. The probe format is versatile allowing the sensor material to be added to aqueous materials, including those for microfluidic cell culture. Water-soluble probes have been primarily used for *in vivo* biological imaging, but they could potentially be applied to other environments in microfluidic devices. An image of this sensor format is shown in **Figure 8**.

There are several disadvantages to water-soluble probes. The lack of an encapsulated matrix creates a higher likelihood of the sensor interfering with the environment of the microfluidic-based hypoxia chamber. It is also more difficult to control the sensor parameters, such as its sensitivity and oxygen selectivity, without an encapsulation matrix. To improve this detection method, water-soluble probes may be bounded to albumin or other molecules to improve the sensor characteristics.

Water-soluble probes are used to monitor dissolved oxygen concentrations in microfluidic devices, usually with cell cultures, in the entirety of the microfluidic channel. One advantage of these probes is it allows techniques such as tomographic imaging to map 3-D images of oxygen concentrations within the cell culture area. However, this isn't a necessity in the Ogle lab and would require more expensive probes molecules, resulting in a higher cost. Reusing the probes would also be impractical. Nevertheless, water-soluble RTDP has been used to detect dissolved oxygen in microfluidic channels in other labs, so we should consider this design format.

Oxygen Sensing Indicators

In conducting our initial research on possibilities for indicator options, we found a large array of available photochemicals that detect oxygen. Further research showed that ruthenium-based indicators and metalloporphyrin-based indicators are the most widely used chemicals for oxygen detection in microfluidic devices designed for cells (Grist). These compounds were compared using their photochemical properties of quantum yield, photostability, and unquenched lifetime. Quantum yield is the ratio of photons absorbed by a molecule to photons emitted back and is used as a measure of the emission efficiency of the fluorescent molecule. Photostability is a measure of how well the compound maintains its accuracy after multiple excitations, and unquenched lifetime, which is the shelf life of the compound. Since the indicator could come in direct contact with the cells, we also investigated cytotoxicity of the compounds.

Ruthenium-based Indicators

There are several ruthenium-based compounds that have been used extensively to detect oxygen. The most common of these indicators are ruthenium-tris-4,7-diphenyl-1,10-phenanthroline ($[Ru(dpp)_3]^{2+}$) [16], ruthenium(II)-tris(1,10-phenanthroline) ($[Ru(phen)_3]^{2+}$) [17], dichlorotris (1,10-phenanthroline) ruthenium(II) hydrate [18], and ruthenium tris (2,2′-dipyridyldichloride)hexahydrate ($[Ru(bipy)_3]^{2+}$), shown in **Figure 9**. Ruthenium-based indicators can be modified to be soluble in polymer films and have previously been used in biological devices. These compounds tend to have a very high quantum yield and good photostability, but they do not remain in their excited state long enough for precise fluorescent lifetime analysis. This short lifetime makes ruthenium-based indicators very inaccurate in low oxygen concentrations [20]. Since the microfluidic device is meant to hold the contained cells in hypoxic (low oxygen) conditions, ruthenium-based indicators are not ideal for our testing. The compounds are easily excitable by LED sources [11]. Analyzing potential cytotoxic effects, $Ru(phen)_3^{2+}$ also has been shown to cause membranes of cells to break after repeated excitation. Additionally $Ru(phen)_3^{2+}$ has been observed passing through the cell membrane and entering into the cytoplasm of cells; however the researchers found no toxic effects for $Ru(bipy)_3^{2+}$ [21]. Ruthenium-based indicators would not be ideal for our testing, but there could be derivatives in this class of compounds that would be acceptable.

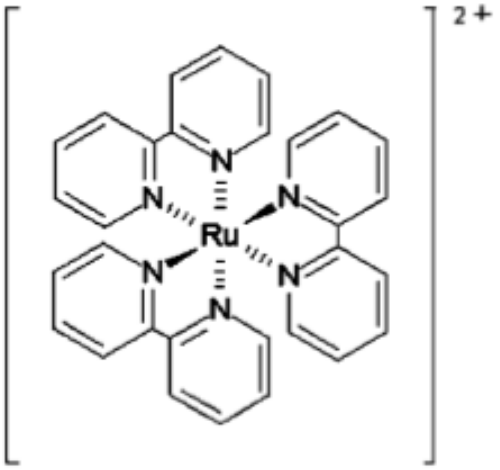


Figure 9. A molecule of a common ruthenium-based indicator [22].

Metalloporphyrin-based Indicators

Many variations of complexes involving a porphyrin ring have phosphorescent properties and are useful for detecting oxygen concentrations. Some of the most common indicators of this class of indicators are complexes of an octaethyl-porphyrin ring with palladium(II) or platinum(II) (OEP) [11]. **Figure 10** shows a generic water-soluble metalloporphyrin compound with the metal complexing at the center of the porphyrin ring. These compounds, like ruthenium-based indicators, have previously been used successfully in numerous biological applications. PdOEP and PtOEP have long luminescent lifetime, due to phosphorescence rather than fluorescence; however, they tend to have low quantum yield and low photostability [11]. The long luminescent lifetime and high makes testing very accurate in

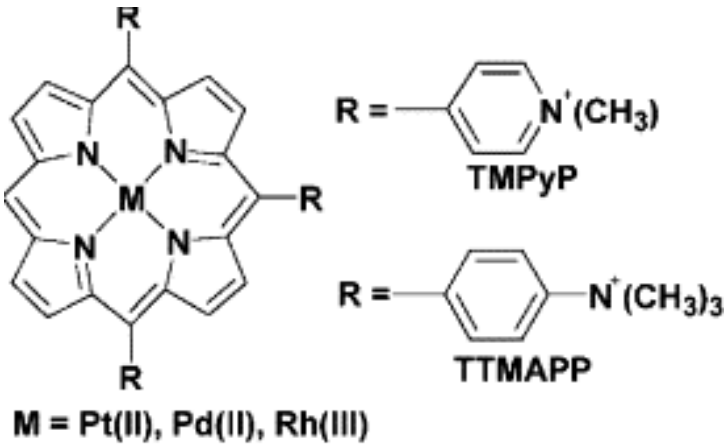


Figure 10. A typical metalloporphyrin molecule [25].

hypoxic conditions. Complexes involving octaethyl-porphyrin ketone (OEPK) have significantly improved photostability and can withstand 18 hours of continuous UV exposure with an absorbance loss of 12% compared to the OEP complex absorbance loss of 90% [23]. This makes PdOEPK and PtOEPK significantly more suitable than OEP complexes for our testing since it will be necessary to take multiple measurements of the gradient created in the microfluidic device. The enhanced photostability and long luminescent lifetimes make PdOEPK and PtOEPK ideal for both intensity-based measurements and lifetime-based measurements. OEPK complexes show very little leaching from these matrices making them less likely to come in contact with the cells and interact with the cellular environment [24]. This limits potential cytotoxic effects of the indicators. Due to their high accuracy in hypoxic conditions, high photostability, and low risk of interaction with the cells in the device, metalloporphyrin indicators, specifically PdOEPK or PtOEPK, seem to be good options for the detection method in the device.

DESIGN MATRICES

Upon ample research into various oxygen detection methods, it was concluded that our design would best be served by analyzing two distinct divisions of the design, oxygen sensor format and indicator. Thus, we created two different design matrices to analyze each part of the design separately.

Oxygen Sensor Format Design Matrix

Table 1. Design Matrix for Oxygen Sensor Format

Oxygen Sensor Format			
Factors	**Thin-Film Sensors**	**Microparticle/ Nanoparticle Sensors**	**Water-Soluble Macromolecule Probes**
Accuracy (30)	4	5	2
Cost (25)	3	3	1
Ease of use (20)	5	4	3
Ease of Assembly (15)	4	3	4
Biocompatibility (10)	5	4	2
Total Points	**81**	**78**	**45**

Above is our design matrix for the three different oxygen sensor formats. We chose to five factors to consider when choosing which design format we should use for our oxygen detection work.

First, we decided to consider accuracy and sensitivity, because this is the main goal of the project, and hence is weighted the most in our design matrix. We want to detect, accurately, how much oxygen is in the microfluidic device channels to see whether or not the fusion of mesenchymal stem cells with the heart after a heart attack (hypoxic conditions) is possible. Because such low levels on oxygen are being used, accuracy is

extremely important to ensure that the sensor can detect as small a change in the oxygen concentration as possible. Through ample research, we concluded that the microparticle/nanoparticle sensor would be the most accurate because of the presence of an indicator encapsulation matrix and the intracellular measurements, giving this format a score of 5. We then ranked thin-film sensors as a close second, assigning a score of 4, due to the fact that it too contains an encapsulation matrix and has been used by many other lab groups that have been able to make a standard curve for the experiments and obtain reliable results. We ranked the water-soluble probes last, quite a bit worse, a score of 2, than the other because it lacks an encapsulated matrix, which increases the chances of sensor interference with the cell environment.

Next, we considered cost: a factor that is considered in a large percentage of design projects. Our client wanted to keep our budget as low as possible because the devices will be used numerous times each day. We estimated that the microparticles/nanoparticles were about the same cost as thin-film sensors, so we gave those formats a score of 3. We then assigned the water-soluble/macromolecule probes a score of 1 because they were the most expensive of the proposed methods.

Then, we determined that the ease of use was also very important for the Ogle lab, because the experiment need to been done frequently and had to be repeatable. The easier to make the microfluidic devices with the sensor could lead to more consistent results and would allow Dr. Ogle and her research team to dedicate more focus on the experimental results. Thin-film sensors seemed to have the most straight forward experimental procedure, while the water-soluble and macromolecule probes seemed to be the most complex, with addition steps such as adding probes in the middle of the experiment and the possibility to correct results for relatively high error compared to that of thin-film sensors and microparticles/nanoparticles. We then concluded that using microparticles/nanoparticles was of middle difficulty falling somewhere in between the two other formats in our design matrix.

Ease of assembly is also important since experiment requires a new device with a new sensor. The procedure to make the beads for the microparticles/nanoparticles seemed to be the most complex, using the most material, giving it a score of 2. While both the thin-film and the water-soluble and macromolecule probes seemed slightly easier to assembly, which is why we ranked those formats one number higher than the microparticles/nanoparticles.

Finally, biocompatibility is a factor because this device will be used to run experiments with cells. This factor, however, isn't of high priority for our client because such testing is so far into the future of the device, and hence was weighted lower. Water-soluble and macromolecule probes are the most likely to interfere with the cells and hence has a very low biocompatibility and scored a 2. The thin-film sensors do not react with the cell cultures because the indicator is farther away than the other two formats, and has an encapsulation matrix, and hence has a very high biocompatibility score of 5. Microparticles/nanoparticles have a slightly lower biocompatibility because it has an

encapsulation matrix, but is also in closer contact to the cells and hence has a higher chance of cell-sensor interactions. Thus we scored this method a 4.

Oxygen Sensing Indicators

Table 2. Design Matrix for Oxygen-sensitive Luminescent Material

Oxygen-Sensitive Luminescent Materials		
Factors	**Ruthenium-based**	**Metalloporphyrin-based**
Luminescence Properties (25)	5	3
Accuracy/ Sensitivity (30)	2	5
Unquenched Lifetime (10)	2	4
Cost (25)	4	2
Biocompatibility (15)	3	5
Total Points	**67**	**73**

For our indicator design matrix, we considered several factors that define an acceptable indicator for integration into the hypoxia microfluidic device. We weighted the potential indicator's sensitivity to oxygen the highest since the ability to detect an oxygen gradient in the microfluidic chamber is the main focus of this semester's project. We also weighted detection properties, such as luminescent lifetime, quantum yield, and photostability, and cost of the chemical significantly as well. Detection properties enable the detection method to work properly and are critical to accurately measuring the gradient in the chamber, and cost, as mentioned in the product design specifications, is to be kept as low as possible by the client's request. A secondary focus of this project is to allow the gradient to be measured while cells are present in the microfluidic device; however, after correspondence with the client, biocompatibility was weighted significantly lower since it is not the main focus of the semester and is not essential to project completion. Similarly, unquenched lifetime, the amount of time a molecule retains its accuracy when it is not in use only holds a small amount of weight because it is not vital to the success of the project.

Due to their inability to sense low oxygen concentrations, ruthenium-based indicators were rated significantly below metalloporphyrin-based indicators in sensitivity to oxygen. Metalloporphyrin-based indicators have very high sensitivity to oxygen and received a perfect score. Metalloporphyrin-based indicators have a higher cost per mole of chemical than ruthenium-based indicators, so ruthenium-based indicators were given a higher score for cost. Because metalloporphyrin-based compounds have significantly longer unquenched lifetimes than ruthenium-based compounds, they received a higher rating, but not a perfect score because they do eventually lose their luminescent properties. Due to the cytotoxicity of $Ru(phen)_3^{2+}$ after repeated excitation, the ruthenium-based indicators were scored lower than metalloporphyrin-based indicators in biocompatibility; however,

other ruthenium-based indicators exist that do not exhibit cytotoxic effects, so the ruthenium-based indicators' score was only reduced to three, while. Metalloporphyrin-based indicators scored perfectly in biocompatibility due to their inability to escape a polymer matrix and interact with the cells in the device.

FINAL DESIGN

Oxygen Sensitive Indicator Selection

Metalloporphyrin-based Material Selection

After analyzing the design matrix for the oxygen-sensitive luminescent materials, a metalloporphyrin-based material was selected. Specifically, platinum (II) octaethylporphyrinketone (PtOEPK) was selected due to its high accuracy, increased photostability, and decreased cost. In general, palladium based metalloporphyrin materials have shown longer phosphorescence lifetime, and thus higher oxygen sensing, than platinum based materials [26]. However, due desirable optical properties, compatibility with readily available and inexpensive solid-state electronic components, and cost, PtOEPK was selected over PdOEPK. PtOEPK is a metal-ligand complex with the chemical formula $C_{36}H_{44}N_4OPt$ and the molecular weight of 743.30 g/mol [27]. The PtOEPK molecule demonstrates strong room-temperature phosphorescence with a high quantum yield and long lifetime [26].

Mechanism of Metalloporphyrin Catalyzed Oxidations

PtOEPK's sensitivity to oxygen is driven by the strong affinity of O_2 to transition metal centers especially with porphyrin ligards. Ultimately, Pt exhibits pro-oxidative actions and photo-oxidation by reducing electron density of the porphyrin ring [26]. It has been found that direct reaction of O_2 with a metalloporphyrin form types of protein-free $L_nM(porp)O_2$ complexes [29]. Attempts to form $L_nM(porp)O_2$ species at ambient conditions results in the formation of bridged oxo species or bridged peroxo-species (CO). The mechanism of the oxidation of the metalloporphyrin molecule is as follows:

$$L_nM + O_2 \underset{}{\overset{O_2}{\rightleftharpoons}} L_nM\text{-}O_2 \overset{L_nM}{\rightleftharpoons} L_nM\text{-}O_2\text{-}ML_n \quad (1)$$

Equation 1. One mechanism for the oxidation of a metalloporphyrin by direct reaction with O_2 [30].

Oxygen Sensor Format Selection

After analyzing the sensor format design matrix, the thin-film sensor method was selected. This method was chosen primarily due to its ease of use and assembly in combination with high accuracy. Because this project's devices will be used with high frequency, ease of use and assembly are highly important. Additionally, thin-film sensors limit interaction with the microfluidic-based hypoxia chamber, significantly reducing possible effects on the operating environment. The thin-film sensor will be comprised of PtOEPK encapsulated in polystyrene. This indicator matrix will then be pipetted onto a glass slide, creating a thin,

even layer. If needed, the matrix can be spin stated on the glass slide to ensure a level, even distribution. The thin-film sensor will be placed under the microfluidic-based hypoxia chamber, as shown in **Figure 11** below.

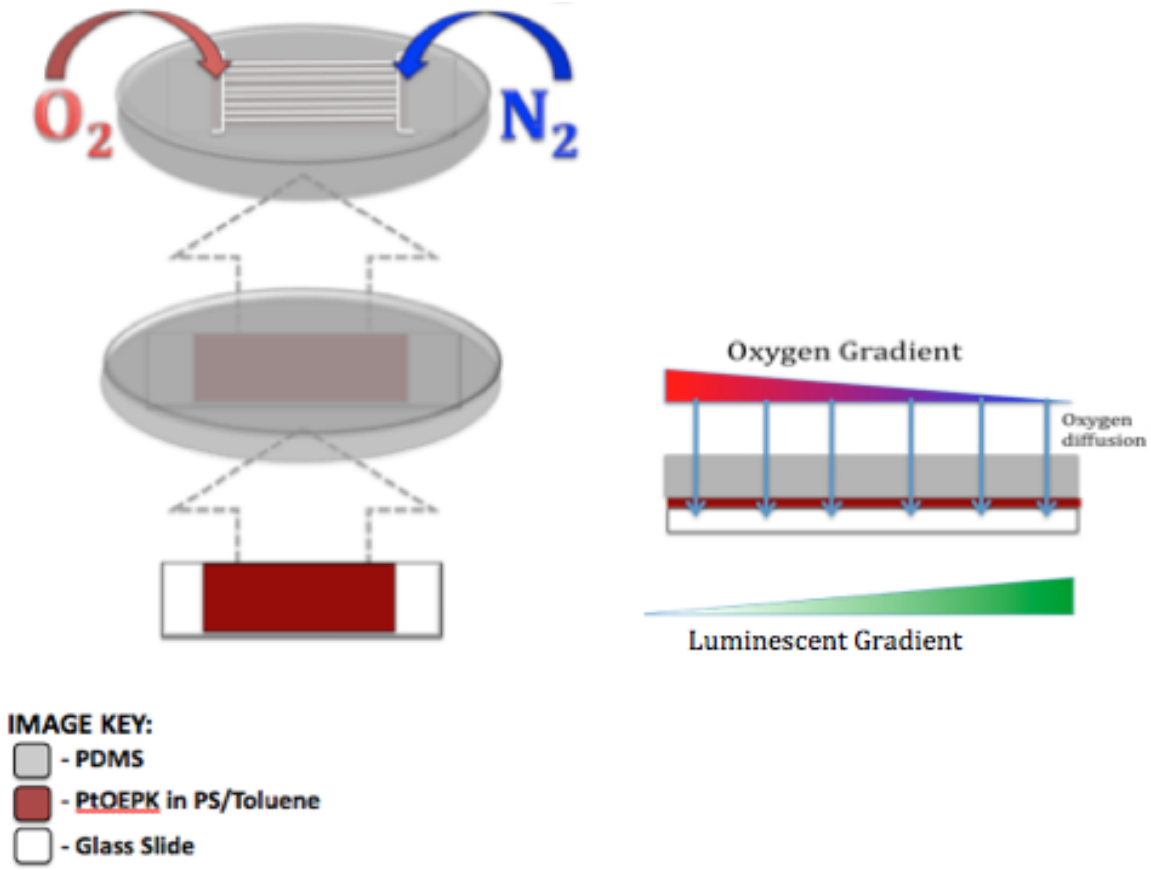


Figure 11. Thin-film oxygen sensor fabricated on a glass slide and placed beneath the microfluidic device.

Method for Detection of Oxygen Gradient

The principle of optical oxygen sensing is founded on dynamic luminescence quenching of luminescent dyes by the presence of oxygen [28]. Luminescence is a process that emits photons. It can be classified as either fluorescence or phosphorescence based on the spin state of the excited state. The material that will be used, PtOEPK, is phosphorescent. Thus, the radiative relaxation of the material will occur from the lowest excited triplet state to the singlet ground state. When in the presence of oxygen, the oxygen molecule (O_2) will act as a quencher, accelerating the decay of the excited state and ultimately reducing intensity [28]. A diagram of this detection method is shown in **Figure 12**.

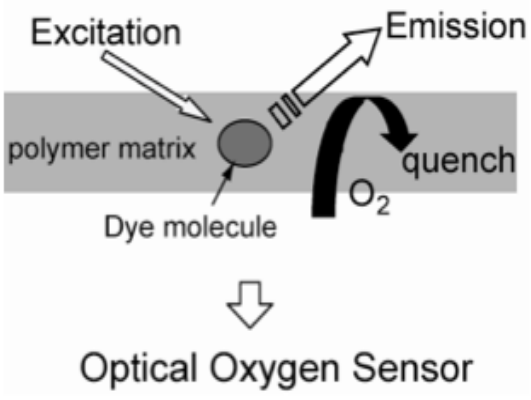


Figure 12. Diagram of how an indicator detects oxygen through an optical sensor [11].

The intensity of PtOEPK thin-film sensor will be measured at conditions exposed to nitrogen, I_0, and oxygen saturated conditions, I_{100}. From the ratio of these intensities, I_0/I_{100}, and known oxygen concentrations, a standardized curve of intensity vs. oxygen concentration will be created. From this data, the Stern-Volmer quenching constant, K_{SV}, can be determined. The Stern-Volmer equation can then be used to relate emission intensity to oxygen concentration [28]:

$$I_0/I = 1 + K_{SV}[O_2] \quad (2)$$

The thin-film sensor will be illuminated using fluorescent lasers. The PtOEPK molecule has absorption peaks of 398 nm 590 nm and an emission peak of 791 nm [7][27], as shown in **Figure 13** below. Literature has shown that oxygen permeable films of PtOEPK-polystyrene demonstrate optimal absorption at 590 nm. Both absorption wavelengths will be tested to ensure the most optimal intensity readings.

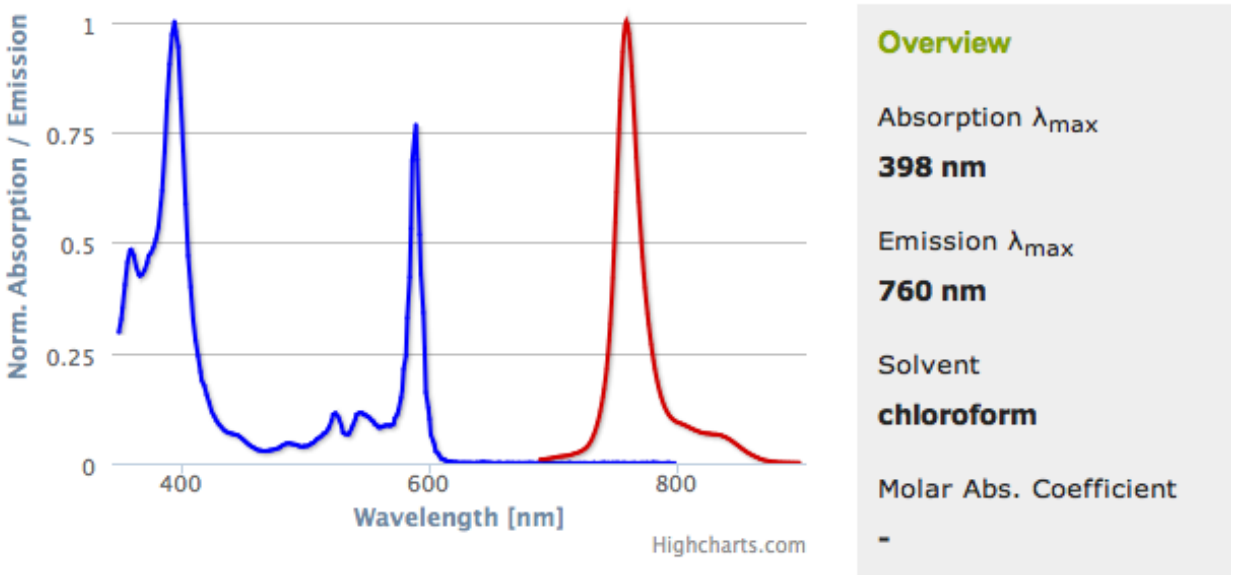


Figure 13. Absorption and emission spectra of PtOEPK [27].

FABRICATION OF DEVICE

The final solution used to create the thin-film sensors was 7% w/w PS in toluene solution, due to its high relative sensitivity and molecular weight. Many research teams have discussed the optimal weight percentage for PS in toluene solution, and have come to the consensus that the 7% w/w ratio of PS to toluene is best [6]. There are many different tests that could have been performed to support this optimal solution, but due to lack of time and materials, the team decided to use the solution deemed optimal by various research sources. Such optimization tests are outlined in the future work section of this paper, as they will be crucial when the thin-film strips are implemented into the final microfluidic-based hypoxia chamber design. The 7% w/w solution is more sensitive and

has a higher rate of change in relative intensities between 0% and 100% oxygen when compared to a 5% w/w PS in toluene solution. This comparison is shown in **Figure 14**.

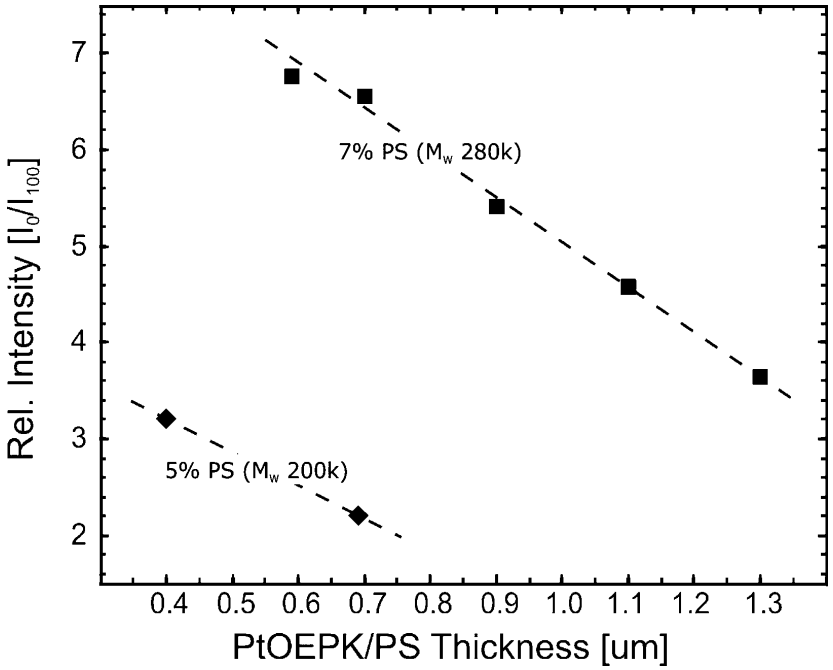


Figure 14. Plot of the sensor intensity ratio vs. film thickness for the two PtOEPK/PS solutions. (I_0 corresponds to 0% and I_{100} to 100% gaseous oxygen, respectively). 1 mg: 1 mL ratio of PtOEPK in encapsulation matrix was used.

Experimental Procedure

Materials purchased for the fabrication of the thin-film sensors include PtOEPK, toluene, and polystyrene. Also, materials used from both the Ogle and teaching labs included four 96-well plates, three 1.5 ml centrifuge tubes, one 50 ml centrifuge tube, a micropipet and tips, a scoopula, four weight boats, a mg-sensitive balance, two 3 ml syringes, two 18 G1 ½ needles, and a vortexer.

To prevent contamination of sensor components during the fabrication process, fabrication was conducted in a sterile environment. To begin, the fume hood was subjected to UV irradiation for 15 minutes. Then, the hood, materials, and gloves were sterilized with ethyl alcohol. To create the encapsulation matrix solution, 1.0g of polystyrene was weight out and combined with 7.102 mL of toluene in a 50-mL centrifuge tube (14%w/w). Next, the PS/toluene mixture was vortexed to speed dissolution of the polystyrene, occasionally breaking up solid coagulates of polystyrene with a pipet tip. After 20 minutes of vortexing, the polystyrene was fully dissolved and ready for PtOEPK to be added. 2.0mg of PtOEPK was massed out into a weigh boat using an analytical balance. Then, 1 mL of toluene was transferred to the weigh boat containing the PtOEPK to dissolve it (2mg/mL). The resulting solution was recovered and combined with 1 mL of the polystyrene in toluene solution in a 15-mL centrifuge tube. This solution is the 'final' thin-film sensor solution, and had a dark red color. With the addition of the excess toluene, this final solution has a 7% w/w PS in toluene. To thicken the solution and ensure proper mixing, the 1.5 ml centrifuge tube was vortexed again. Then, the solution was divided into 32µL aliquots.

These aliquots filled 18 wells on two 96-well plates and were organized as shown in **Figure 15**. After the solution was fully distributed into the well plates, the plates were covered with their lids and stored at room temperature. Parafilm was placed around the lid of the 50 mL centrifuge tube containing the remainder of the PS/toluene solution to prevent toluene evaporation. This tube was then labeled and stored it at 4˚C in the refrigerator in ECB 2005.

Figure 3. Placement of the final PtOEPK/PS-Toluene solution in a 96 well-plate

COST ANALYSIS

Dr. Ogle gave the design team an open-ended budget for this project, but wanted to keep cost to a minimum when deciding upon which materials to purchase. The cost breakdown of the materials that were used to make our oxygen-detecting sensors are shown in **Table 3**. Over the course of the semester, we spent approximately $300, which was fully funded by Dr. Brenda Ogle's lab. A detailed breakdown of this analysis is in **Appendix C**.

Table 3. Expenses for Materials used in Fabrication

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**		

TESTING AND EXPERIMENTATION

Testing for this project consisted of two parts: attempting to make dissolved oxygen solutions to be used for the creation of oxygen concentrations over the thin-film sensors, and imaging testing with the confocal microscope at Bock Labs.

Creating Dissolved Oxygen Solutions

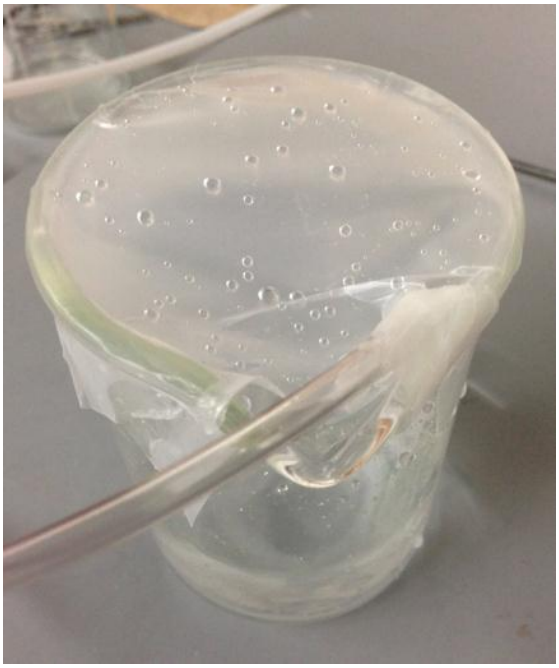


Figure 14. Photo of the set-up used for making dissolved oxygen solutions.

In order to test different oxygen concentrations and establish a standardized curve for the PtOEPK, the team decided to attempt to make solutions of deionized water with various concentrations of dissolved oxygen. There were two main issues to overcome with this concept; the first was how to create solutions of different oxygen concentrations, and the second was how to maintain each solution at its set concentration. The team developed a plan to create solutions of different concentrations by combining different amounts of 100% oxygen and 0% oxygen. Bubbling oxygen gas through water, shown in **Figure 15**, for an extended period of time would create the saturated oxygen solution, and supersaturating the water with nitrogen in the same way would create solution the oxygen deficient solution. These solutions could be combined in relative quantities to give relative percent oxygen solutions. The second issue dealt with preventing the air from contacting the solutions once the team made them. The team attempted several methods including plugging wells in the well plate, sealing the wells with Parafilm, and using a self-sealing vial, but none of these were compatible with imaging and effective at sealing the solution from the air. The team ultimately abandoned this aspect of the project due to time constraints, focusing instead on simply whether the material detects oxygen.

Imaging Procedure

After developing the thin-film sensors, the team looked into imaging devices that were available for use. The Ogle lab is equipped with a fluorescence imaging system, so it was concluded that imaging would be done with fluorescence instead of luminescence. This ensures that future work on this project can be done with the resources already available to the Ogle lab team. Because it is difficult to reserve scopes at other labs and make time to learn other systems, the project's efficiency is greatly increased by using the Ogle lab's equipment. Literature states that PtOEPK can be imaged under either system, and that intensity and lifetime are both viable testing options [6].

To test whether the indicator indeed detects oxygen, a Nikon Eclipse Ti microscope with a 20X objective lens magnification was used to image the sensors. This microscope is located

in Bock Labs, and is part of the University of Wisconsin-Madison's Laboratory for Optical and Computational Instrumentation (LOCI). There was not sufficient time to set up oxygen and nitrogen pumps in Bock Labs, so testing was only performed for one oxygen concentration. Thus, the thin-film sensors were tested at atmospheric oxygen conditions, which contain approximately 21% oxygen.

Different lasers were used to excite the thin-film strips in order to determine which absorption peak was best for exciting PtOEPK. Additionally, a photomultiplier tube (PMT) was used to detect the light our indicator emitted. Together, this system was used to read intensity of the signals from PtOEPK. Such signals were read by the Prairie View V.4.3.1.22 program. In order to find PtOEPK sites, intensity readings were tested when moving the scope around the 96 well-plate. **Figure 16** shows a typical intensity reading from the confocal microscope, where the white areas on the graph represent intensity signals.

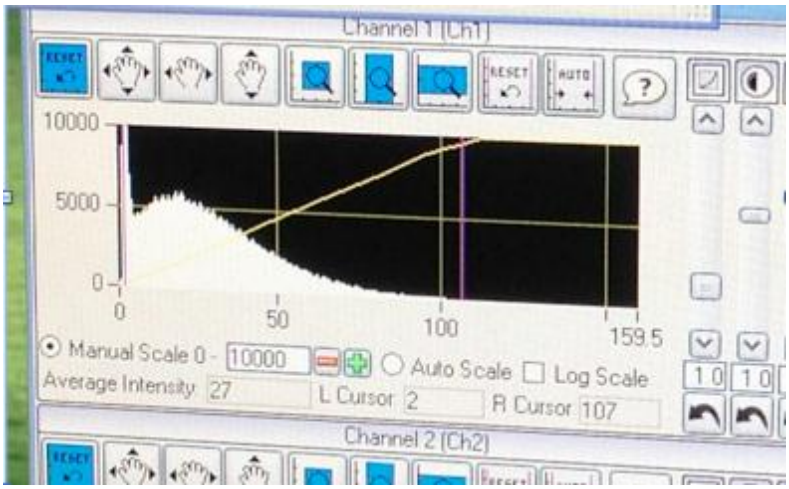


Figure 15. Excitation graph of the Prairie View V.4.3.1.22 program during the imaging of PtOEPK/PS.

Both wells with and without the thin-film sensors were imaged so that the data contained a control value to compare the device to. Wells with the thin-film sensors were imaged with a 405 nm laser because this was the closest laser wavelength to our first excitation wavelength of PtOEPK, 398 nm [11]. Next, wells were tested with a higher laser frequency, 561 nm, to excite PtOEPK at the second excitation wavelength, 590 nm in polystyrene [11].

RESULTS

When a well containing the thin-film sensors was imaged with a 405 nm laser, a significant excitation of PtOEPK was observed (an intensity of approximately 130). On the other hand, when an empty well was imaged, the average intensity reading was 7, which is most likely due to noise interference from the adjacent wells containing PtOEPK. This image from the empty well is shown as image (a) in **Figure 16**. The wells containing thin-film sensors showed a comparable difference the empty well, as indicated by the bright white spots in both images (b) and (c) in **Figure 16**.

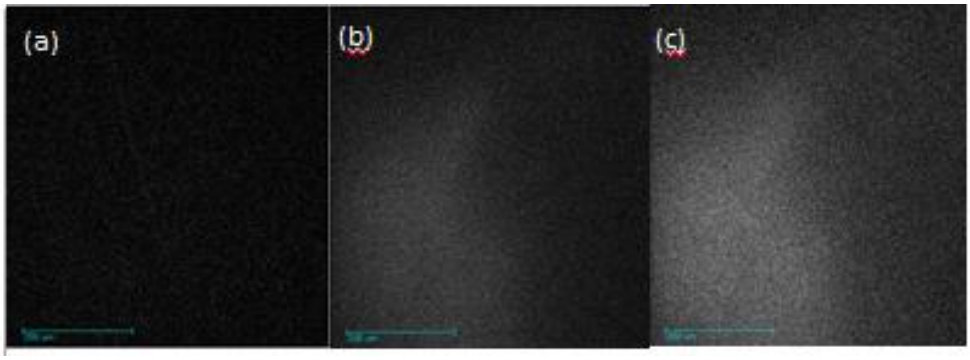
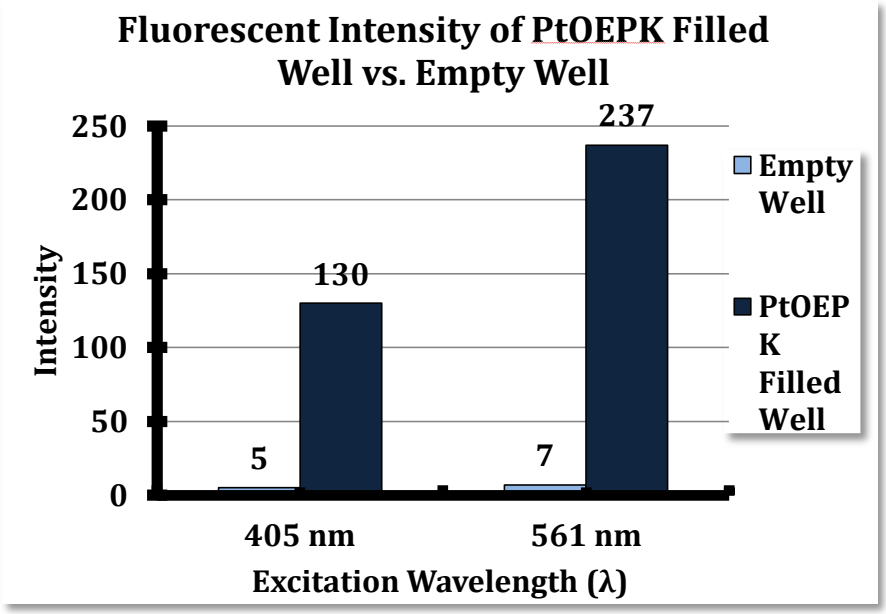


Figure 16. Fluorescent intensity of (a) an empty well, (b) a sensor well excited with a 405 nm laster, and (c) a sensor well excited with a 561nm laser

Additional tests were performed using a 561 nm laser to excite the PtOEPK in the thin-film sensors. When this was performed, a brighter excitation was observed in the material than was observed when using the 405 nm laser. This is most likely because the 561nm laser excited PtOEPK at its second excitation wavelength of 590 nm [6]. With this higher excitation wavelength, the average intensity was found to be 237. Image (c) in **Figure 16** shows the image that was produced with the 561 nm excitation laser.

The various intensity signals and their associated excitation wavelengths are shown in the graph below, which summarizes the results from initial imaging at Bock Labs.



DISCUSSION

Fabrication of Device

During initial fabrication of thin-film sensors, it was discovered that the polystyrene purchased does not have polymerization inhibitors. Consequently, when PtOEPK was

added to the PS/toluene solution, the solution became very viscous. This was due to the polystyrene interacting with the reactive platinum (II) atom in PtOEPK. Because the polystyrene solution was pipetted directly into the weigh boat containing the PtOEPK during our initial fabrication attempt, this viscosity led to significant problems recovering the final solution from the weigh boat. To correct this issue, we doubled the concentration of the polystyrene solution (initially 7%w/w) and the PtOEPK (initially 1mg/mL) allowing us to suspend the PtOEPK in toluene without polystyrene present. Then we combined equal amounts of the two solutions to result in a solution with the correct final concentrations (7%w/w polystyrene in toluene with 1mg/mL PtOEPK). Viscosity was also an issue when pipetting the solution into the well-plates. To compensate, we shortened the end of the pipet tip with a scissors, thereby widening the opening and making it easier to work with such a viscous solution. In addition to the issues with making the solution, the first test films that we fabricated were not evenly distributed in the bottom of each well. The matrix solution was thinner in the center of the well than around the side. To correct this, we changed our aliquot amount from 30μL per well to 32μL per well. With these issues resolved, subsequent attempts at fabrication were much more efficient and effective.

Imaging Testing and Results

The imaging results aren't conclusive to the accuracy of our indicator to detect oxygen at varying concentrations; they merely prove that the PtOEPK-containing thin-film sensors can indeed detect oxygen as they were designed to do. Further testing will need to be conducted with different oxygen concentrations in order to determine if the thin-film sensors are viable for detecting varying oxygen concentrations. Specifically, they will need to be tested with numerous trials at hypoxic oxygen concentrations, 1% to 21% oxygen, to ensure that they can be utilized with the microfluidic-based hypoxia chamber. A high degree of precision and accuracy is required for these results to deem the design successful and ready for implementation.

Additionally, the intensity readings that were taken with the confocal microscope were lower than expected because the filter wasn't completely compatible with PtOEPK. Because the microscope used a 690/60 filter and the peak emission wavelength of PtOPEK is 760nm, some of emission spectra was cut off during testing. Thus, the images were not as bright as they might be with the use of a proper long pass filter. The best filter to be used with PtOEPK is a 720/60 nm filter, which is currently being ordered by Bock Labs. With the use of this filter, more of the emission spectra will be picked up, and it is predicted that the intensity readings will be higher, producing brighter images.

CONCLUSIONS

Upon analyzing the data produced during imaging of the thin-film sensor well-plate, it is concluded that the fabricated thin-film sensors detect oxygen at atmospheric concentrations (approximately 21%). Thus, the design is successful at initial testing steps, and has fulfilled.

FUTURE WORK

New Equipment and Materials

In order to perform the aforementioned testing, it is necessary to find a long pass filter that can be used with either the Ogle lab's microscope, the Ashton lab's microscope, or the confocal microscope at Bock Labs. This filter should be a 720/60 filter so that it can detect the emission wavelength of 760nm. Professor Kevin Eliceiri of LOCI has stated that he can set up either the Ogle or Ashton lab's fluorescent imaging microscope with such a filter so that proper imaging of the sensors can be completed. The team suggests that if Dr. Ogle and her team want to continue with using PtOEPK as their luminescent material in oxygen-detecting sensors, they should have a 720/60 long pass filter set up in their own lab. This will increase efficiency, and will quickly produce results for the progression of this project.

Another purchase that would better the functionality of our thin-film sensors is higher-grade polystyrene. As mentioned in the **DISCUSSION** section, it was determined that the polystyrene purchased from Sigma Aldrich did not contain polymerization inhibitors. This was discovered during initial fabrication trials when the PS/toluene/PtOEPK solution became extremely viscous, making it difficult to transfer into our wells. After reading into the exact grade of polystyrene that was purchased, there was no indication that it contains polymerization inhibitors. So, it is recommended that the next set of thin-film sensors are created with analytical grade polystyrene that contains polymerization inhibitors. Although this will be a higher cost, it will allow for production of more optimal sensors.

Optimization of Thin-Film Sensors

Another future aspect of this project is optimization of the thin-film sensors. This will include fabricating multiple sensors that have different weight percents of the PS/toluene encapsulation matrix, varied ratios of PtOEPK, and varied thickness of the strips. These different tests are outlined in **Table 4**.

Table 4. Optimization Tests

Test	% w/w PS in toluene	mg of PtOEPK for 1ml encapsulation matrix
1A	1	0.1
1B	1	1.0
1C	1	10
2A	7	0.1
2B	7	1.0
2C	7	10
3A	17	0.1
3B	17	1.0
3C	17	10

During initial fabrication of the thin-film strips, the strips were created with the specifications listed for test 2B. Those specifications were deemed most optimal in the research that was performed. However, in order to ensure the most efficient use of our

materials, we should perform these optimization tests ourselves. However, tests 1C, 2C, and 3C most likely will not be performed because they use a large amount of PtOEPK, which is quite expensive. Testing must be performed with each type of sensor in order to determine which is optimal. Such tests will be the same as described above, and standardized curves will be created for each test case.

Developing Standardized Curve

Upon initial testing to ensure that the sensors in fact detect oxygen at varied oxygen concentrations, more tests are necessary in order to create a standardized curve. These tests will be performed using the sensors that were made in the well-plates, and varied oxygen concentrations will be created using a hypoxia chamber from either the Ashton lab or Ogle lab. The use of a hypoxia chamber ensures that the oxygen concentrations in the wells are accurate, which will allow us to make Stern-Volmer plots of the test data. This standardized curve will be used during the implementation of the strips with the microfluidic device.

The intensity of PtOEPK thin-film sensor will be measured at conditions exposed to nitrogen, I_0, varying oxygen concentration, and oxygen saturated conditions, I_{100}. From the ratio of these intensities, I_0/I_{100}, and known oxygen concentrations, a standardized curve of intensity vs. oxygen concentration will be created. By plotting the intensity vs. oxygen concentration values and creating a best fit line to the plot, the Stern-Volmer quenching constant, K_{SV}, can be determined. The slope of the best-fit line is the Stern-Volmer quenching constant. The Stern-Volmer equation can then be used to relate emission intensity to oxygen concentration [28]:

$$I_0/I = 1 + K_{SV}[O_2] \quad (2)$$

This equation will be combined with the intensity data, will be used to determine oxygen concentrations and to create a standardized curve for the thin-film sensors.

Integration with Microfluidic Hypoxia Chamber

The next steps of this project entail combining the thin-film strips with the microfluidic device created by Dr. Ogle's previous design team. In order to do this, the sensors will be fabricated directly onto glass slides, and will be spin-stated in order to ensure an even surface. The microfluidic devices will then be placed directly on top of the oxygen-detecting strips. The thin-film sensors will then be ready for any kind of experiment in which oxygen needs to be detected in the microfluidic channels. Such experiments, performed by Dr. Ogle's team, will aim to discover the best oxygen concentrations for which stem cell fusion will occur. The oxygen-detecting strips will ensure that these experiments are performed under accurate oxygen concentrations and that results will be precise.

ACKNOWLEDGEMENTS

Our team would like to formally thank our client, Dr. Brenda Ogle for presenting us with this design project and assisting us throughout the entire design process. Additionally, we would like to acknowledge Brian Freeman, a graduate student working in the Ogle lab, for contributing beneficial advice and assistance on our design. Brian was crucial towards the end of the semester when we faced struggles with backordered products. Also, we thank Drew Birrenkott, an undergraduate student also working in the Ogle lab, for showing us how the microfluidic devices are produced and work, as well as contributing his own research into oxygen detection methods to our design.

Our advisor, Dr. Randolph Ashton also deserves many thanks for guiding us through the entire design process, and reviewing our progress to ensure its high quality. Dr. Ashton was also vital to our project during the imaging stages when we didn't have access to a proper imaging system. He went out of his way to find us a system that is compatible with our materials, and ultimately led us to a successful contact. We would like to thank this contact, Professor Kevin Eliceiri, director of the Laboratory for Optical and Computational Instrumentation (LOCI). On short notice, Professor Eliceiri was able to schedule us some time with the confocal microscope at Bock Labs for imaging of our sensors. We would also like to thank Julie Last, for assisting us during imaging of our sensors at Bock Labs.

Lastly, we would like to acknowledge the Biomedical Engineering Department at the University of Wisconsin – Madison for giving us the opportunity to expand our engineering experience through the BME 200/300 design course.

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APPENDIX A

Generation of an Accessible and Versatile Hypoxia Chamber
Product Design Specifications
October 19th, 2012

Client: *Professor Brenda Ogle, PhD*

Advisor: *Professor Randolph Ashton, PhD*

Team: *Matthew Zanotelli*
Chelsea Bledsoe
Karl Kabarowski
Evan Lange

Function:

Microfluidic devices have been proposed to improve accessibility, versatility, and to generate overall function of hypoxia environments. The purpose of this project is to design and produce a microfluidic-based hypoxia chamber in which cells (cardiomyoctes and mescenchymal stem cells) can be cultured and exposed to varying, controlled concentrations of oxygen. Previous work on this design project has produced a functioning microfluidic-based hypoxia chamber. Thus, this semester's work will focus on testing the functionality of the device and developing a means to accurately monitor and detect the varying oxygen concentrations and gradients present in the chamber. This device will ultimately be used to facilitate experimental investigations involving oxidative stress, ischemic, and reactive oxygen species (ROS)-mediated cellular pathways.

Client Requirements:

- Accurate and reliable oxygen detection method that is capable of determining oxygen concentrations at specific points in the microfluidic-based hypoxia chamber
- Oxygen detection method that is able to detect oxygen concentrations from 21% O_2 (ambient concentration at room temperature) to 1% O_2
- All components of the oxygen detection system need to be biocompatible with the microfluidic-based hypoxia chamber and with cell culture in general
- All components and chemicals used for detection need to be non-toxic to cells
- Ability to be used frequently with a high level of repeatability
- Price of device should be as low as possible to allow routine use

Design Requirements:

1. **Physical and Operation Characteristics**

    a. *Performance Requirements*: The oxygen detection device needs to accurately detect and measure oxygen concentrations present in the microfluidic-based hypoxia chamber both when cells are present and when they are not. Consequently, the device will need to measure oxygen gradients in cell culture

media and must have no negative effects on culturing cells. Ideally, the device will have the ability to be used multiple times, as well as detect and measure oxygen concentrations fluorescently.

b. *Safety*: All materials in the device must be safe for handling under basic laboratory safety procedures. The device should be in compliance with mammalian cell culture standard operating procedures. Luminescent material/chemicals need to be non-cytotoxic and not rupture cell plasma membranes when illuminated.

c. *Accuracy and Reliability*: This device will need to have a great deal of precision (repeatability) and accuracy in the detection and measurement of oxygen concentrations. The system needs to function within a range of +/- 2-3% oxygen concentration and needs to be able to detect oxygen concentrations from 21% O_2 (ambient concentration at room temperature) to 1% O_2. Additionally, there should be no more than +/- 10% error among different tests in order to ensure repeatability and to allow precise comparison of experiments performed.

d. *Life in Service*: The life in service of the detection system will be determined by the microfluidic-based hypoxia chamber it will be used with. Each microfluidic-based hypoxia chamber is designed to last through one experiment. This experiment will run no longer than two weeks with an average experiment time of three to four hours (amount of time for hypoxic conditions to be observed in cardiomyoctes).

e. *Shelf Life*: The device should be able to function accurately for approximately one year, so that it can be used for a multitude of experiments and stored for future use. Once in use, the device must persist and maintain accurate functionality throughout an entire experiment and work effectively in the presence of cell culture media and cells.

f. *Operating Environment*: The oxygen detection device will be used in an incubator to create an environment (37°C and 5% CO_2) that mimics facets of the in-vivo environment of cardiac cells in an in-vitro system and bathed in standard cell culture media (DMEM). When cell culturing is performed, the system will be exposed to 2500 Pa in the microfluidic gas channels. For imaging and analysis, a fluorescent microscope will be used. During fluorescent microscopy, the device should be expected to handle a 24-hour time-lapse and intense fluorescent exposure lasting up to 3 hours in duration.

g. *Ergonomics*: The oxygen detection device should be easy to use, in order to ensure a high level of repeatability among different users. The device should be able to be used with limited experience, as well as by different and multiple users.

h. *Size*: The size of the oxygen detection device should be relatively reflective of the size of the microfluidic-based hypoxia chamber (approximately 75mm x 25mm).

In order to interact with cells cultured in the hypoxia chamber, the device will also need to fit into the cell channels, which are 250-500μM tall and 250-750μM wide. The device will also need to fit onto a fluorescent microscope of imaging and analysis.

i. *Weight*: The weight of the device should be kept to a minimum in order to maximize ease of use and efficiency; however, weight is not critical in this design and is a low priority consideration.

j. *Materials*: The materials of the device should be able to interact with the microfluidic-based hypoxia chamber with no negative or inhibitory effects. Furthermore, the materials should have no negative effects on cells and need to be non-cytotoxic. Ideally, the components and chemicals used for oxygen detection would have fluorescent properties.

k. *Aesthetics, Appearance, and Finish*: The device should provide clear and distinct indications of the presence oxygen and display distinct changes in oxygen gradients. The finish and aesthetics are not critical and are a low priority consideration in the design.

2. Production Characteristics

a. *Quantity*: There should be one oxygen detection device per microfluidic-based hypoxia chamber.

b. *Target Product Cost*: The cost of the device should be kept to a minimum; however, if a novel and repeatable method is developed, a higher product cost will be considered.

3. Miscellaneous

a. *Standards and Specifications*: This device is not drug related, and therefore does not need approval by the FDA for use or testing. Additionally, no animal or human subjects will be used to test the device. However, the device will need to meet mammalian cell culture standard operation procedures and specifications. Oxygen detection must be accurate to +/- 1% oxygen consideration.

b. *Customer:* The device is created for Dr. Brenda Ogle and graduate student Brian Freeman. The overall goal of Professor Ogle's laboratory is to transform the theories of regenerative medicine into clinical practice. The device should be easy to use and repeatable so that other members of the Ogle Lab can use it. The highest priority for the customer is ensuring accuracy.

c. *Patient-related Concerns*: The device will be used with cardiomyoctes and mescenchymal stem cells and will thus need to be sterile for all uses. There are no

concerns regarding data storage or confidentiality with this device, as the subjects are not patients.

d. *Competition*: The University of Michigan and the MacDiarmid Institute for Advanced Materials and Nanotechnology at the University of Canterbury have created thin-sensor films for oxygen detection in microfluidic devices; however, these devices do not offer the easy of use and affordability desired. There are also other thin-film sensor, microparticle/nanoparticle sensors, and water-soluble/macromolecule probes that have been manufactured by research laboratories using a variety of luminescent material. Commercial thin-film sensors are available, but they offer a limited variety in luminescent material used and are often very expensive. Commercial electrodes can be used for oxygen detection; however, they are very inaccurate.

APPENDIX B

Developing an Oxygen Detection Device for a Microfluidic Hypoxia Chamber
Final Design Selection
November 5th, 2012

Client: *Professor Brenda Ogle, PhD*

Advisor: *Professor Randolph Ashton, PhD*

Team: *Matthew Zanotelli*
Chelsea Bledsoe
Karl Kabarowski
Evan Lange

Final Design:

Metalloporphyrin-based thin-film sensor
Luminescent Material: PdOEPK
Encapsulation Matrix: Polystyrene

Thin-Film Sensor Design:

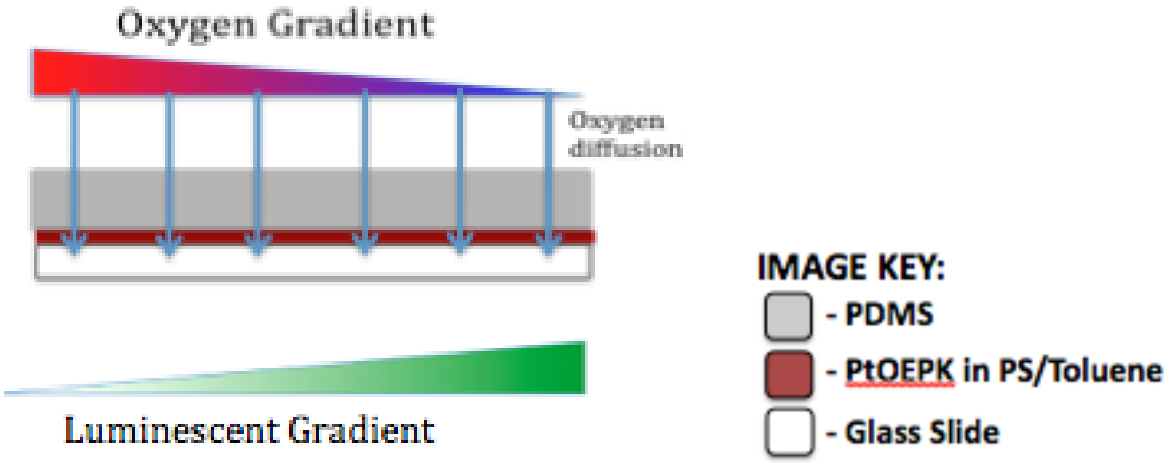


Figure 7. Slide view of thin-film sensor with microfluidic device.

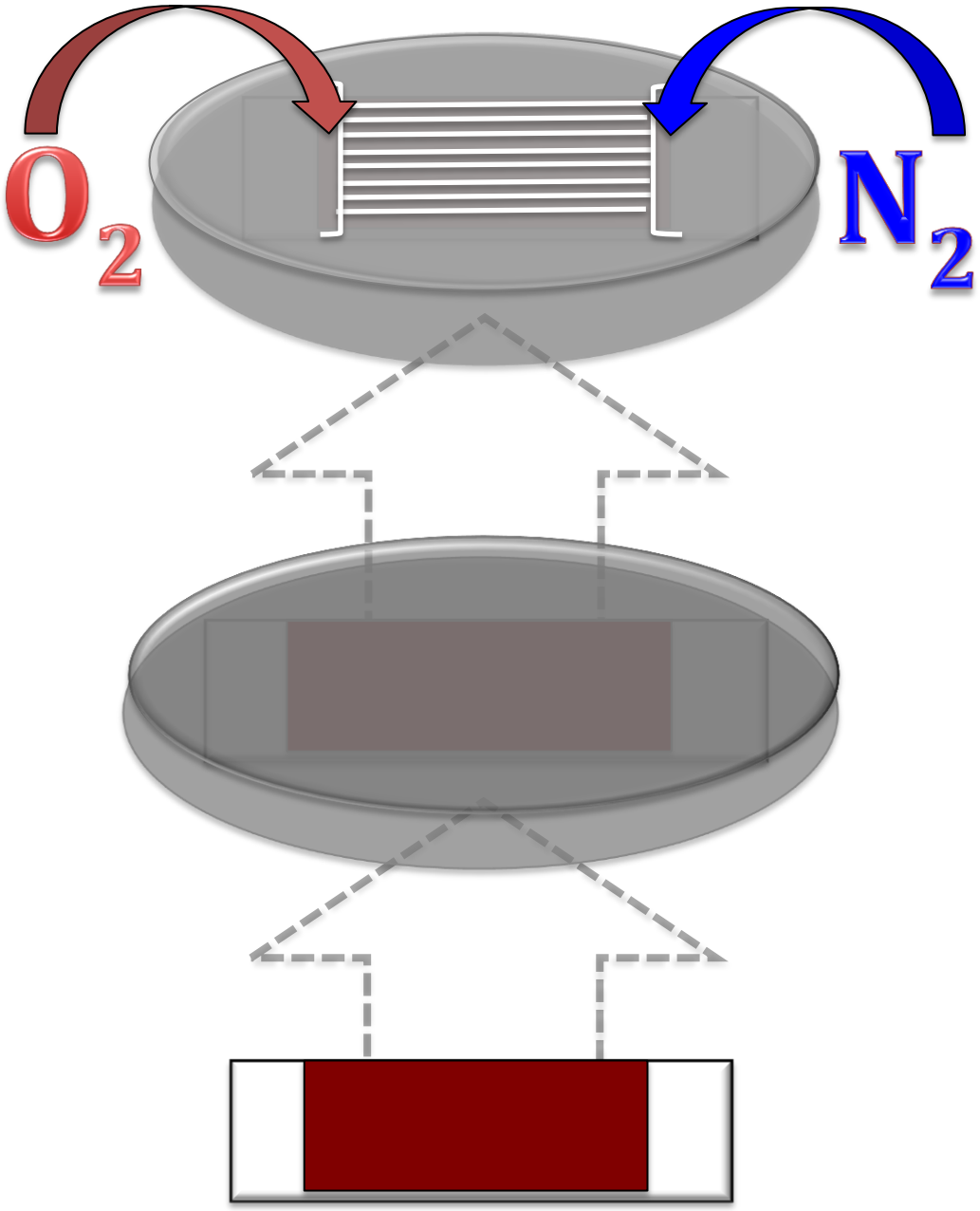
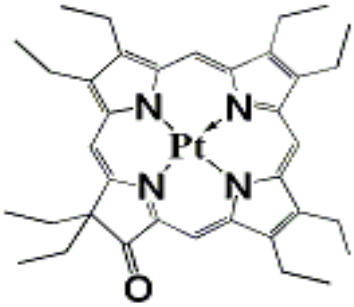


Figure 8. Thin-film oxygen sensor fabricated on a glass slide and placed beneath the microfluidic device for oxygen detection.

Background Information on Luminescent Material:

General Information:



Common name(s): PtOEPK

IUPAC name:	Platinum(II) octaethylporphyrinketone
Chemical Formula:	$C_{36}H_{44}N_4OPt$
Molecular Weight:	743.30 g/mol
Substance Class:	Metal-Ligand Complex

Spectra of

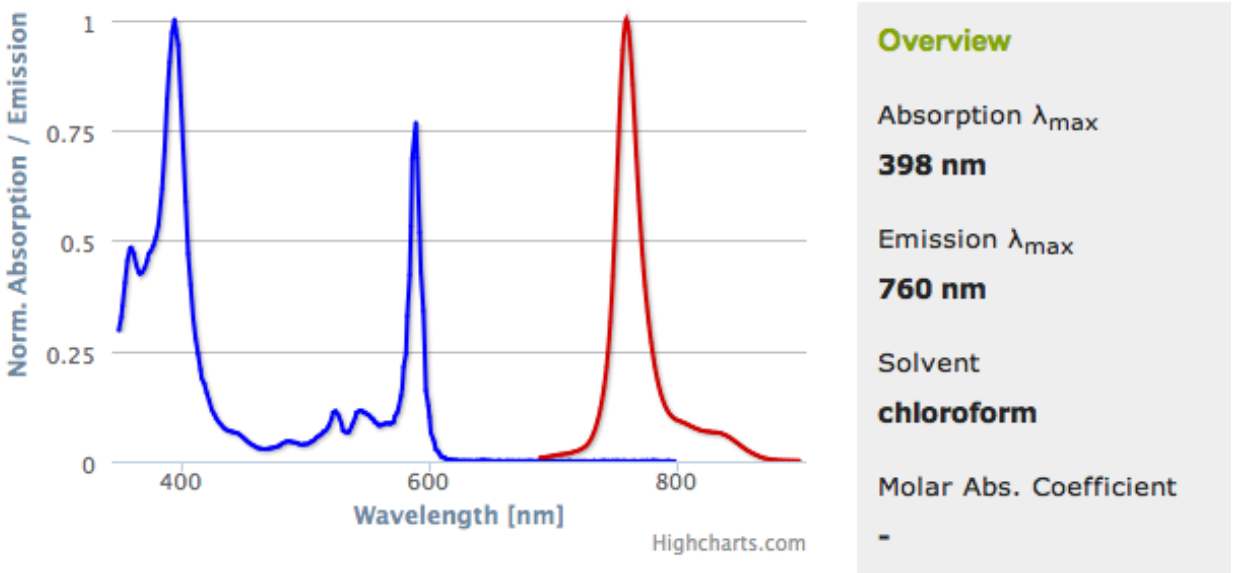


Figure 9. Absorption and emission spectra of PtOEPK. [1].

Compound Qualities

Indicator	Encapsulation Matrix	Unquenched Lifetime (µs)	Quantum Yield	Reported Sensitivity *	Excitation Peaks (nm)	Emission Peaks (nm)
PtOEPK	Polystyrene	61.4 at 22°C	0.12	High	398, 592	759
PtOEPK	PDMA	NR	NR	Q_{DO} = 97.5%	NR	754
PdOEPK	Polystyrene	480 at 22°C	0.01	Very high	410, 602	790

Figure 10. Properties of luminescent material in various encapsulation matrices [2].

Setup for Intensity-bases Optical Oxygen Sensing

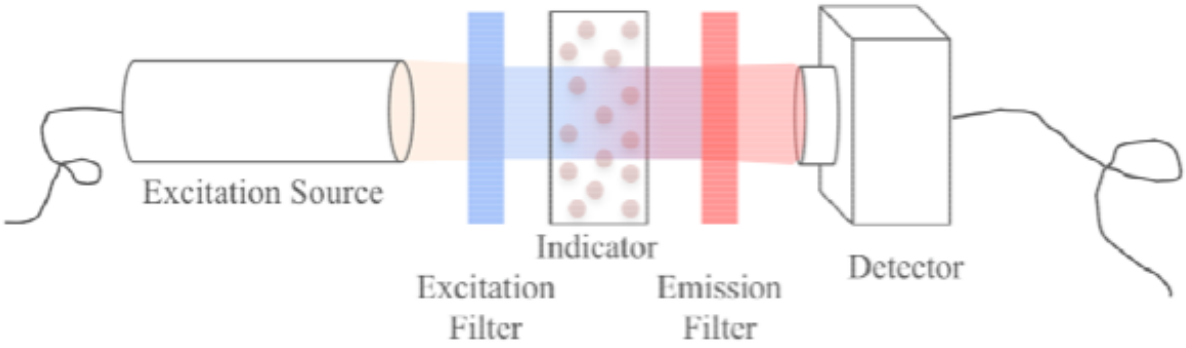


Figure 11. Simplified luminescent imaging system for oxygen sensing used for excitation of indicator material [2]

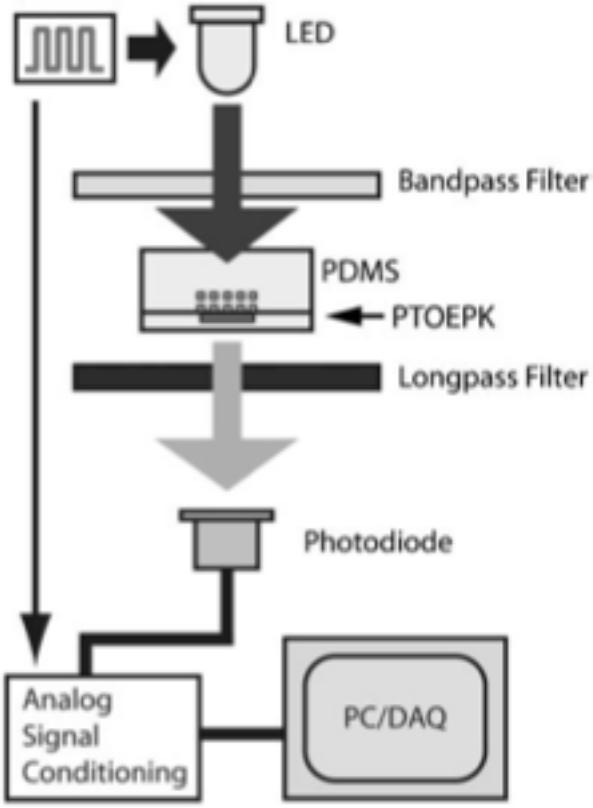


Figure 12. Optical and electrical components used in an excitation-detection system [3].

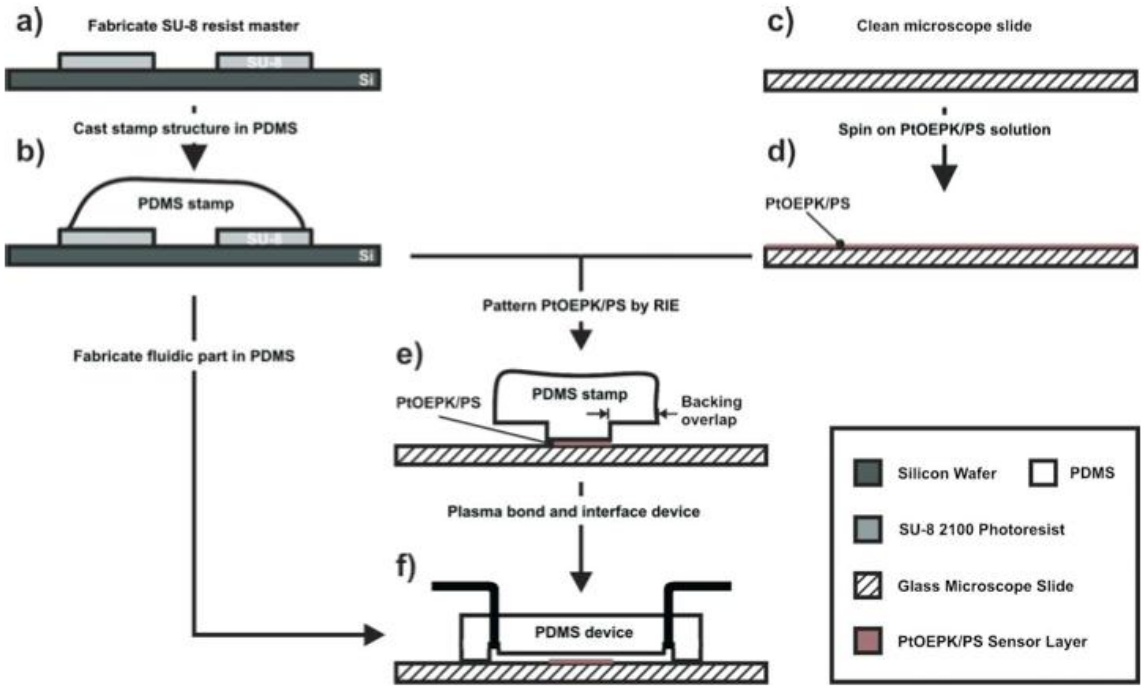


Figure 13. Schematic of the sensor fabrication process showing stamp fabrication in PDMS [4].

References:

[1] **http://www.fluorophores.tugraz.at/substance/633**

[2] Grist S.M., Chrostowski L., Cheung K.C. Optical Oxygen Sensors for Applications in Microfluidic Cell Culture. *Sensors*. 2010; 10(10):9286-9316.

[3] Vollmer, A.P.; Probstein, R.F.; Gilbert, R.; Thorsen, T. Development of an integrated microfluidic platform for dynamic oxygen sensing and delivery in a flowing medium. *Lab Chip* **2005,** *5*, 1059-1066.

[4] Nock V, Blaikie RJ, David T. Patterning, integration and characterisation of polymer optical oxygen sensors for microfluidic devices. Lab Chip. 2008;8:1300–1307. [PubMed]

APPENDIX C

Developing an Oxygen Detection Device for a Microfluidic Hypoxia Chamber
Cost Analysis of Thin-film Sensor
November 16th, 2012

Client: *Professor Brenda Ogle, PhD*

Advisor: *Professor Randolph Ashton, PhD*

Team: *Matthew Zanotelli*
Chelsea Bledsoe
Karl Kabarowski
Evan Lange

Materials:

Material: Pt(II) Octaethylporphine Ketone
Company: Frontier Scientific, Inc.
Catalog No.: 040969
Formula: $C_{36}H_{44}N_4OPt$
Mass: 743.85 g/mol
Options/Sizes: 10 mg
Pricing: $235.00

Material: Polystyrene
Company: Sigma Aldrich
Catalog No.: 182427
Formula: $[CH_2CH(C_6H_5)]_n$
Molecular Weight: ~280,000
Density: 1.047 g/mL
Options/Sizes: 25 G
Pricing: $34.10

Material: Toluene (anhydrous, 99.8%)
Company: Sigma Aldrich
Catalog No.: 244511
Formula: $C_6H_5CH_3$
Molecular Weight: 92.14
Density: 0.865 g/mL
Options/Sizes: 100 mL
Pricing: $28.80

Fabrication of Thin-Film Sensor

- PS pellets dissolved in toluene to yield a 7% w/w solution
    - 7 g Polystyrene in 93 g toluene = 7% w/w solution

- PtOEPK dye added at 1 mg per 1 mL of PS solution
- Films prepared by pipetting 200 μL of solution onto 50 mm X 75 mm glass microscope slides
- Spin state to ensure flat, even surface

Materials Needed for Single Thin-Film Sensor

0.200 mg PtOEPK = $4.70
200 μL 7% w/w polystyrene solution
    12.26 mg Polystyrene = $0.0167
    188.29 μL Toluene = $ 0.054

TOTAL PRICE: $4.77 per thin-film sensor (NOTE: glass slides not included)