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

Mid-semester Report

Biomedical Engineering Design 300/200
University of Wisconsin – Madison
October 23rd, 2012

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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. It is shown that this fusion is more likely to occur under hypoxic conditions, where the cells are introduced to low levels of oxygen. Such conditions are mimicked in in vitro microfluidic-based hypoxia chambers, which create an oxygen gradient across the device. An important component of such devices is detecting accurate oxygen concentrations within the channels to ensure that the cells are truly under hypoxic conditions. Thus, this design project focuses on developing an oxygen detection method for use in the microfluidic hypoxia chamber that was created in the spring of 2012 by the previous design team. The oxygen detection method alternatives consist of three different formats and two indicators. The formats for the sensor include thin-film strips, micro/nanoparticles, and water-soluble macroparticles. Additionally, the indicator alternatives are ruthenium-based and metalloporphyrin-based. After analyzing these designs, a final design of a thin-film sensor with a metalloporphyrin-based indicator was determined. Once the thin-film strips are manufactured, they will be tested and a standardized curve will be produced. Then, the thin-film sensors can be integrated in the microfluidic device, and can successfully detect oxygen concentrations in the functioning hypoxia chamber.

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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].

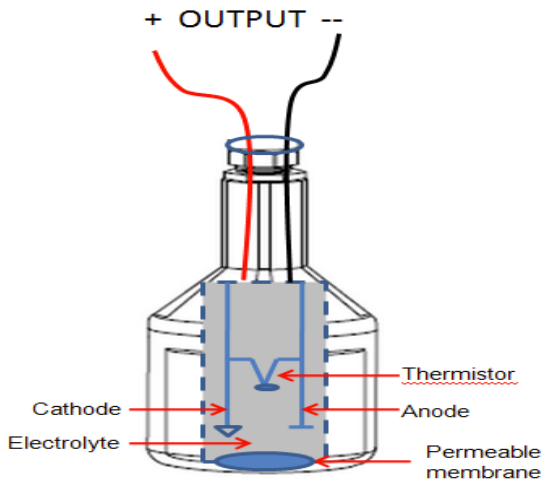

Figure 2. DO6400 Series Dissolved Oxygen Sensor with NI Wireless Sensor Networks (WSN) provided by National Instruments [4].

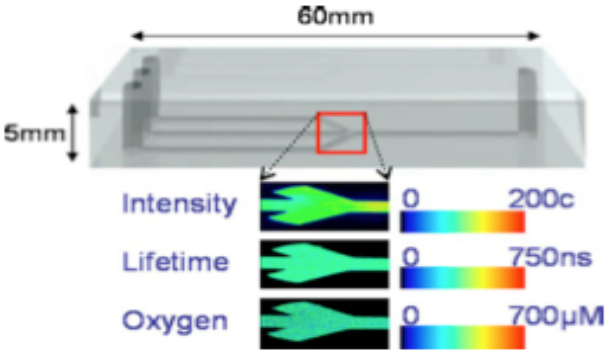

Figure 3. Illustration of fluorescence intensity and lifetime imaging in microfluidic devices using the method developed at the University of Michigan [5].

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 (**Figure 3**) [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 function 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.**

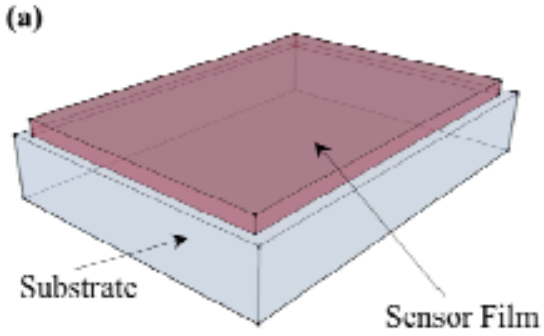

Figure 4. A single thin-film sensor on a generic substrate [11].

For our device, we will most likely pipette the indicator solution 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. Our film sensors will be used to detect oxygen concentration in the channels of the cell cultures.

Fabricated thin layers have also been lithographically patterned using PDMS stamps as shown in **Figure 5** on the following page.

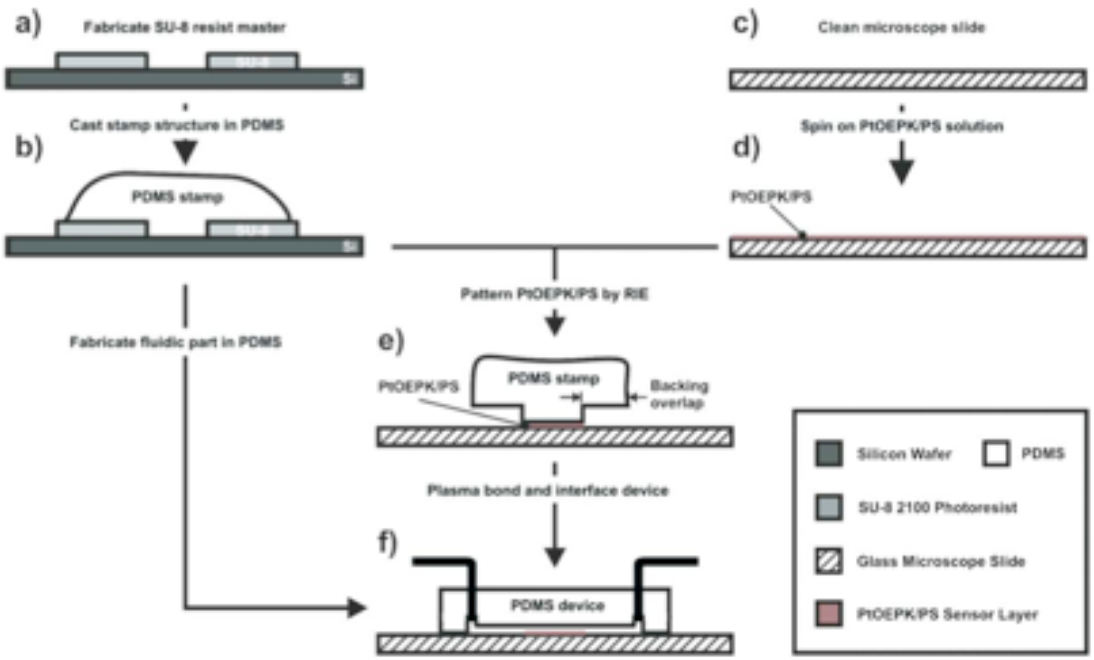

Figure 5. Schematic of the sensor fabrication process showing stamp fabrication in PDMS. [6]

First, 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** below.

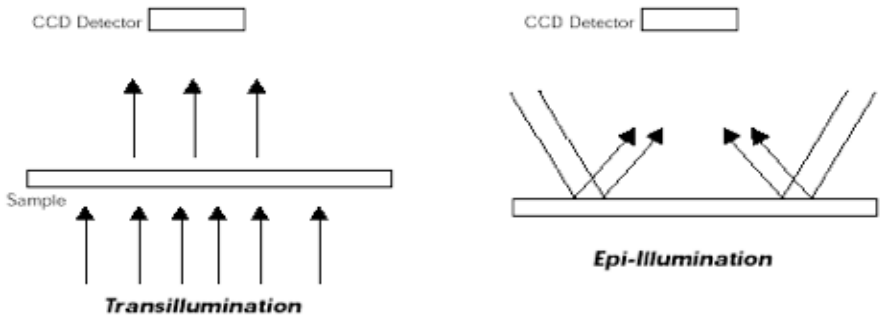

Figure 6. Diagram of the different illumination processes.

In the diagram above, 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** to the right. 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.

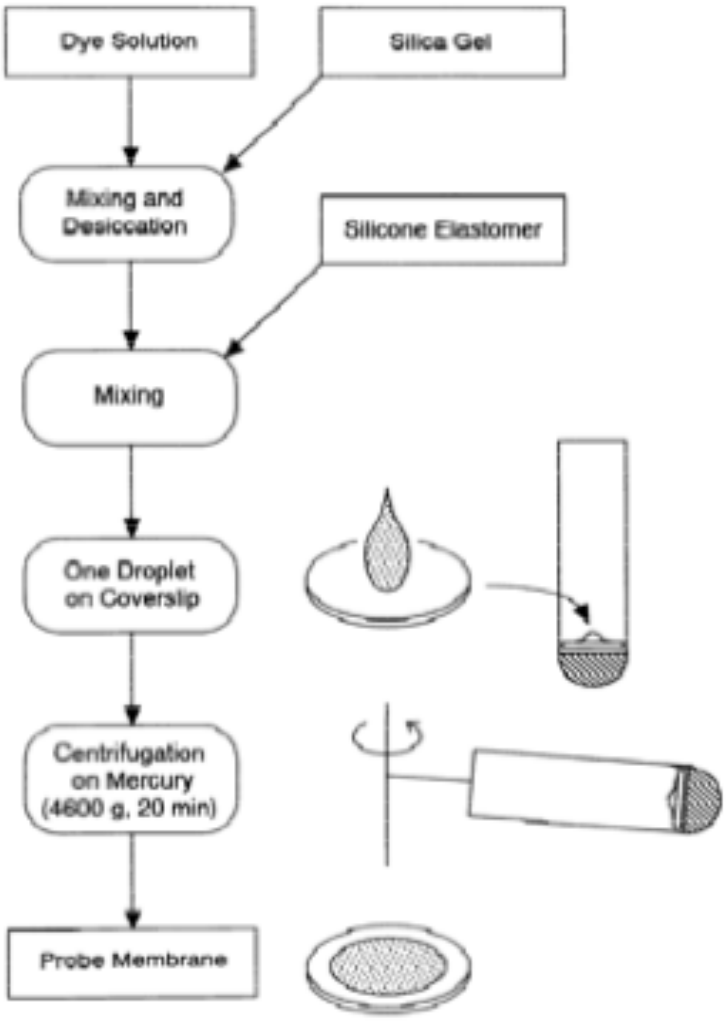

Figure 5. Preparation of oxygen-sensitive probe membrane. This procedure yields a circular membrane that is composed of a thin layer of dyed silica gel beads and a silicon rubber film, supported by a microscope glass cover slip [13].

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.

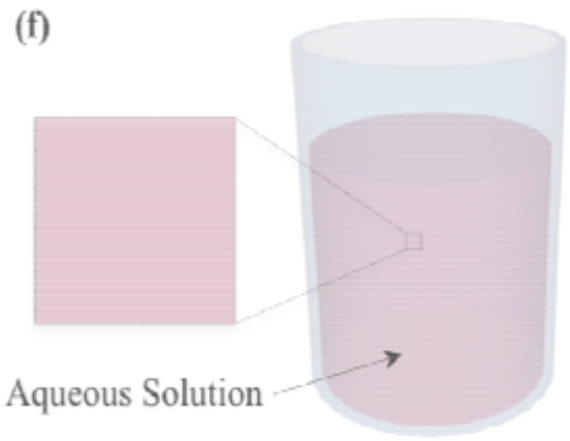

Figure 6. Diagram of water-soluble sensor compound dissolved in aqueous media [11].

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** to the left.

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** [19]. 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.

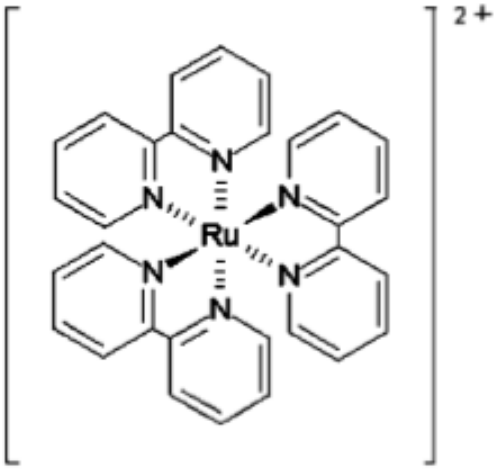

Figure 7. A molecule of a common ruthenium-based indicator [22].

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

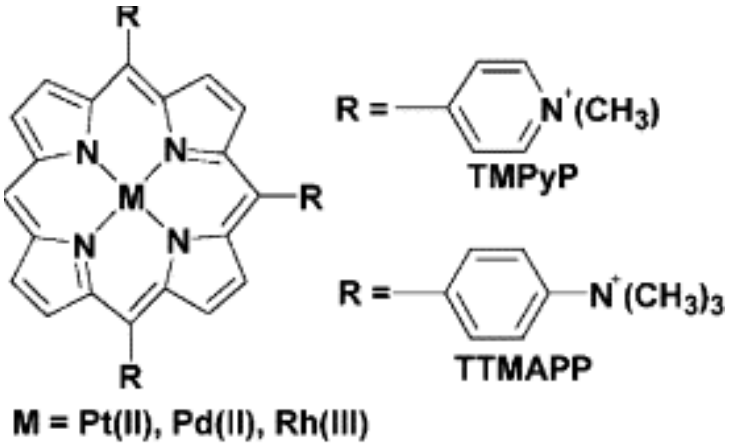

Figure 8. A typical metalloporphyrin molecule [25].

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 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. Oxygen sensor format design matrix.

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. Oxygen-sensitive luminescent material design matrix.

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.

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FINAL DESIGN

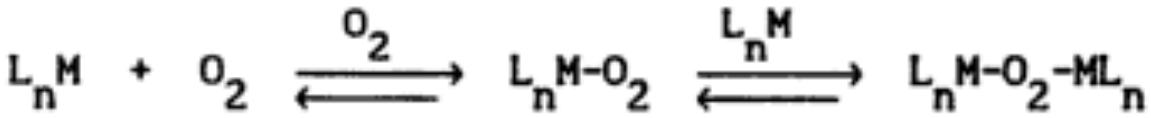
Indicator Selection

Metalloporphyrin-based Material Selection

After analyzing the oxygen-sensitive luminescent materials design matrix, a metalloporphyrin-based material was selected. Specifically, platinum (II) octaethylporphyrinketone (PtOEPK) was selected due to its high accuracy and increased photostability when compared to other metalloporphyrin-based materials. 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 availability, 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 high quantum yield and long lifetime [26].

Mechanism of Metalloporphyrin Catalyzed Oxidations

PdOEPK's sensitivity to oxygen is driven by the strong affinity of O_2 to transition metal centers especially with porphyrin ligards. Ultimately, Pd 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:



One mechanism for the oxidation of a metalloporphyrin by direct reaction with O2 [30].

(1)

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. It also limits interaction with the microfluidic-based hypoxia chamber, significantly reducing possible effects on the operating environment. The thin-film sensor will be comprised of PdOEPK 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.

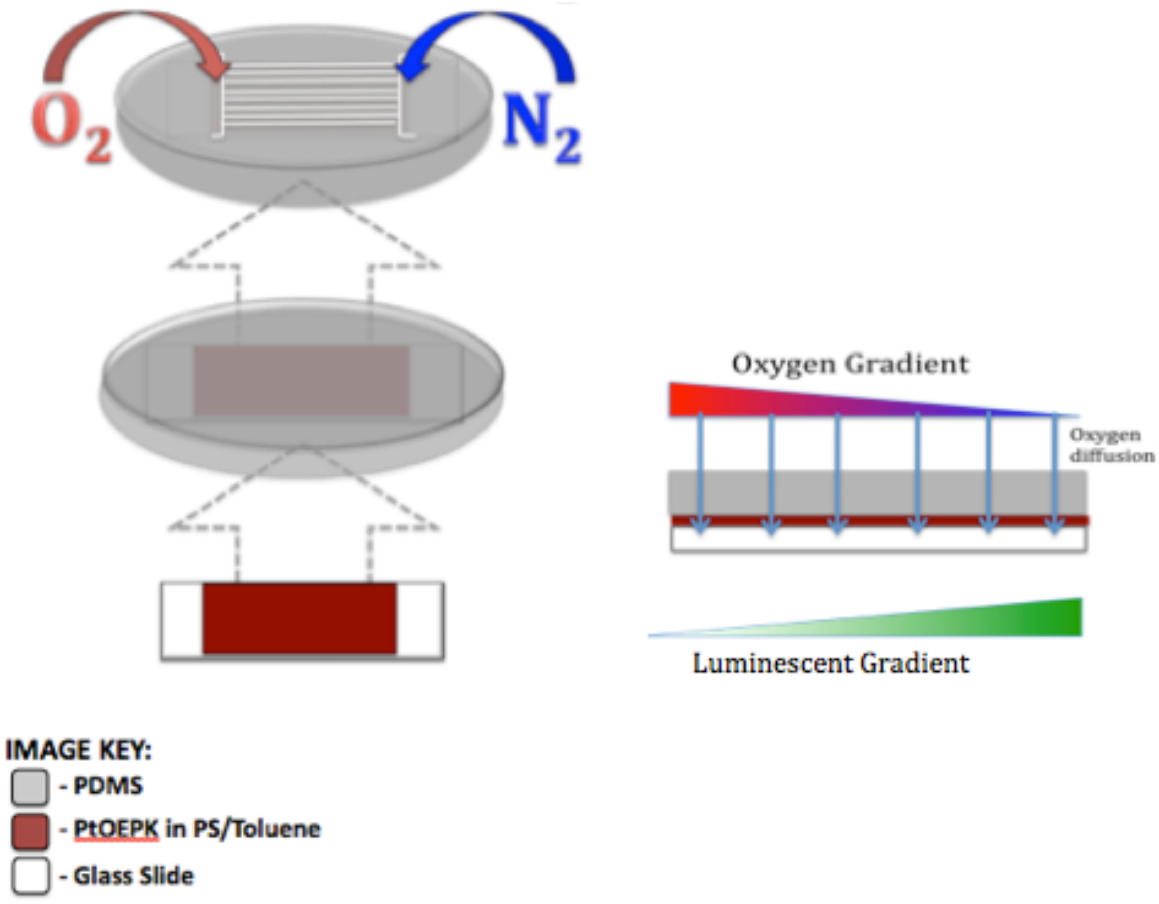


Figure 9. Thin-film oxygen sensor fabricated on a glass slide and placed beneath the microfluidic device for oxygen detection.

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

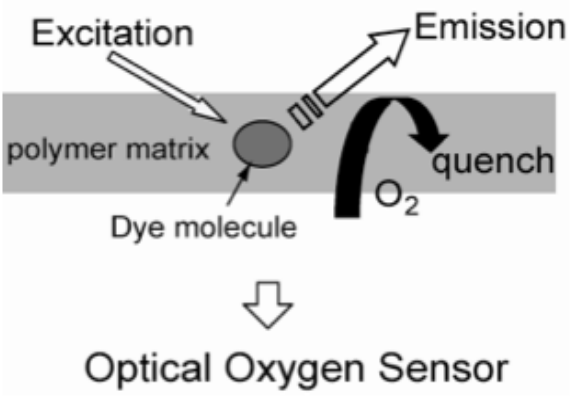


Figure 10. Diagram of how an indicator detects oxygen through an optical sensor [11].

reducing intensity [28]. A diagram of this detection method is shown in **Figure 12**.

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 can 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.

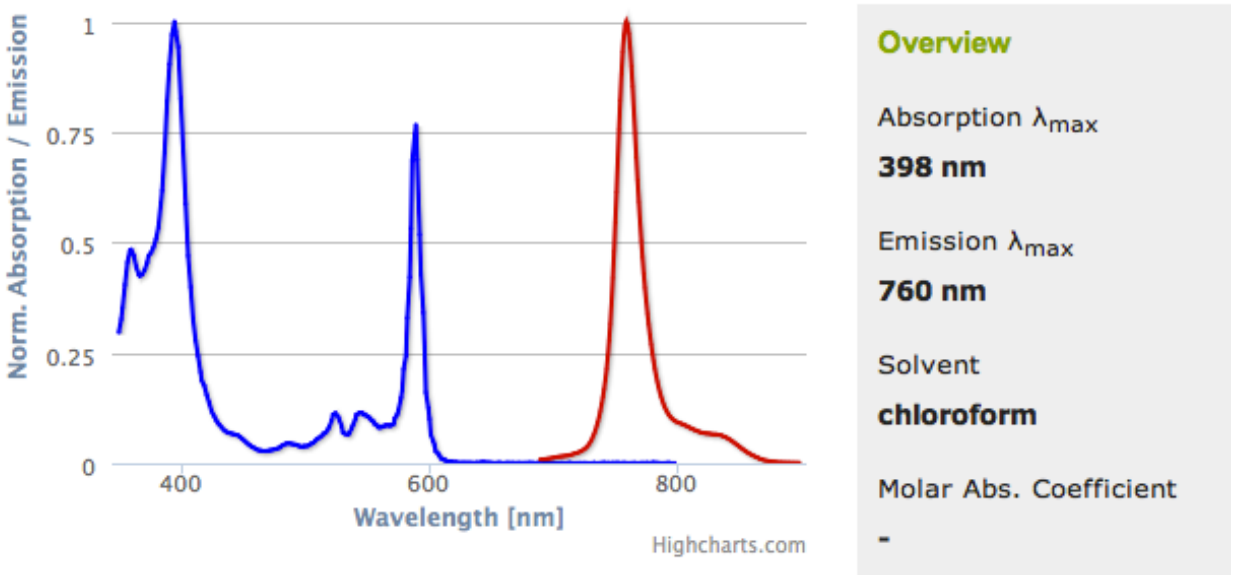


Figure 11. Absorption and emission spectra of PtOEPK [27].

FUTURE WORK

Upon deciding on the final design, the proposed oxygen detection method was presented to the client, who made a few suggestions as to what the next steps should be for the project. First off, the team needs to come up with a cost analysis of the materials that are necessary to produce a bulk quantity of thin-film strips. Since there aren't any commercial thin-film strips that are made with a metalloporphyrin-based indicator. Thus, the strips must be fabricated in the Ogle lab in order for them to be used in the microfluidic devices that have already been manufactured. Once the client approves the cost per slide, the team will purchase materials to be used to manufacture the thin-film strips. The polystyrene and indicator will be purchased through Dr. Ogle, from Sigma Aldrich. Once all materials are gathered, the sensors will be made into the bottom of well plates so that their accuracy can

be tested. After testing, the sensing strips will be made directly onto glass slides so that the microfluidic devices can be placed on top of the sensors, and experiments can be run with the successful hypoxia chambers.

After successfully manufacturing the thin film sensors, they will need to be tested in order to determine their accuracy. In order to test the strips, they don't necessarily need to be integrated with the microfluidic devices themselves. Thus, well plates will be used to test the effectiveness of the thin-film strips. In order to do this, the sensors will be placed on the bottom of individual wells, and the entire well plate will be used in a larger-scale hypoxia chamber to produce a specific oxygen concentration environment for the wells. Then, the wells will be sealed and tested under a fluorescence scope to determine the oxygen concentration in them. This data will be used to formulate a standardized curve that can be used to ensure accuracy when the strips are integrated into the microfluidic devices.

Once the thin-film sensing strips are deemed an accurate oxygen detection method while being integrated with the microfluidic devices, the hypoxia chamber will be used to test cells under hypoxic conditions. Experiments will aim to discover the best methods that allow stem cell fusion to occur. The proposed oxygen detection method will ensure that the experiments are accurate and are being carried out under the most precise standards.

ACKNOWLEDGEMENTS

Our team would like to formally thank our client, Dr. Brenda Ogle for presenting us with this design project and assisting in the desing 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. 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 first half of the semester, and reviewing our work on the way to ensure its high quality. 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 Conceptual Diagram
October 19th, 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:

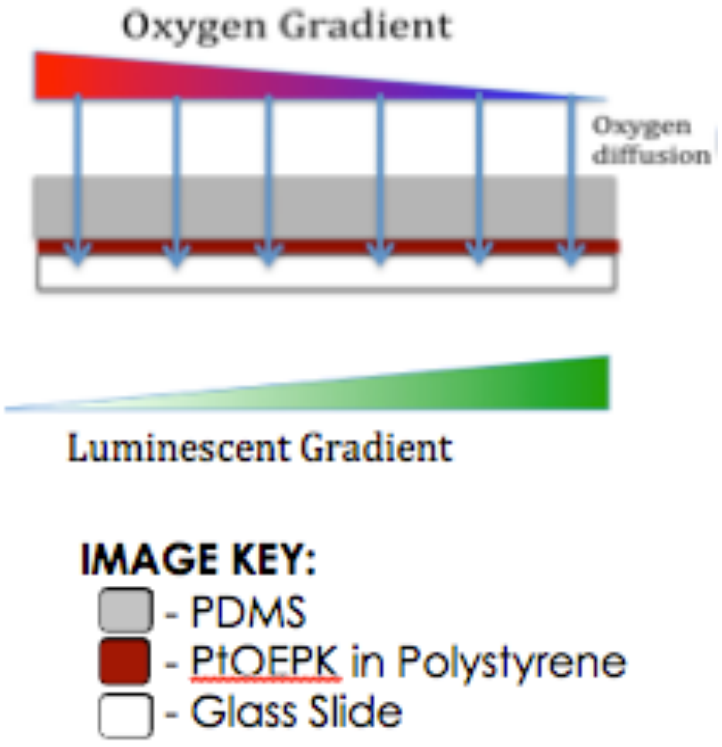


Figure 12. Slide view of thin-film sensor with microfluidic device.

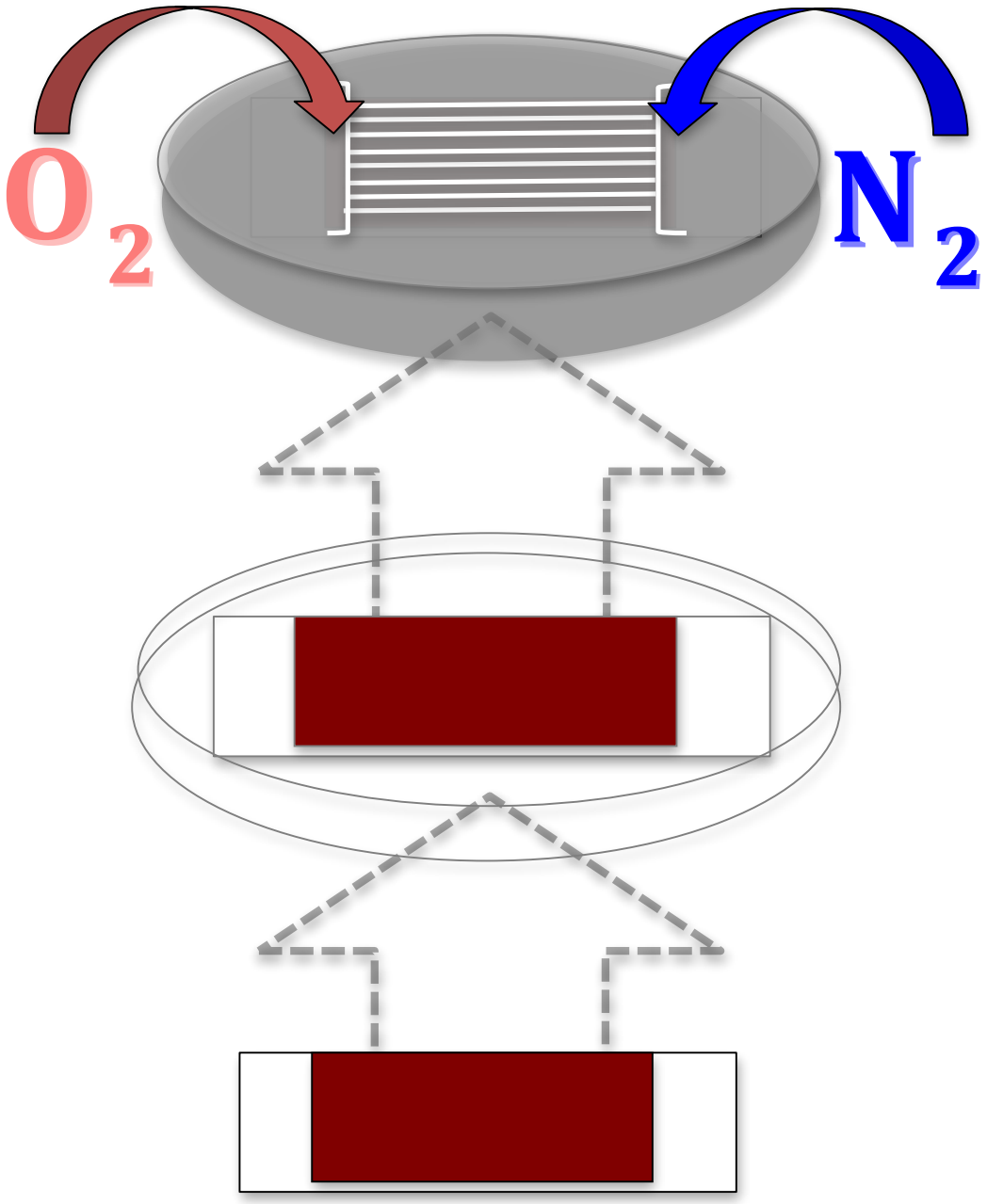
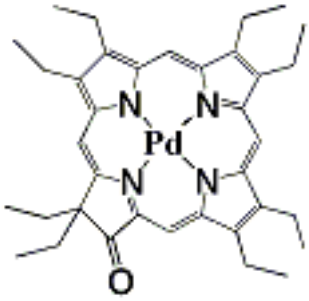


Figure 13. Thin-film oxgyen sensor fabricated on a glass slide and placed beneath the microfluidic device for oxygen detection.

Background Information on Luminescent Material:

General Information:



Common name(s): PdOEPK

IUPAC name:	palladium(II) octaethylporphyrinketone
Chemical Formula:	$C_{36}H_{44}N_4OPd$
Molecular Weight:	654.00 g/mol
Substance Class:	Metal-Ligand Complex

Spectra

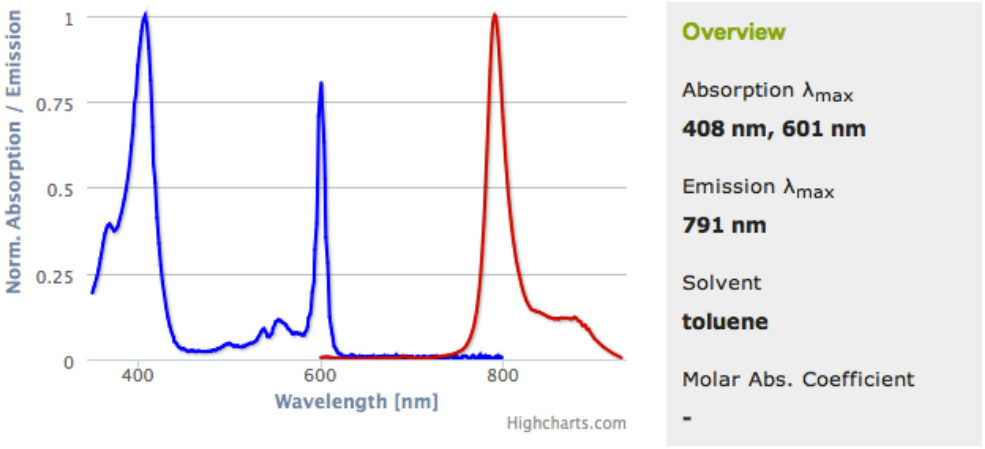


Figure 14. Absorption and emission spectra of PtOEPK. [1].

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

Setup for Intensity-bases Optical Oxygen Sensing

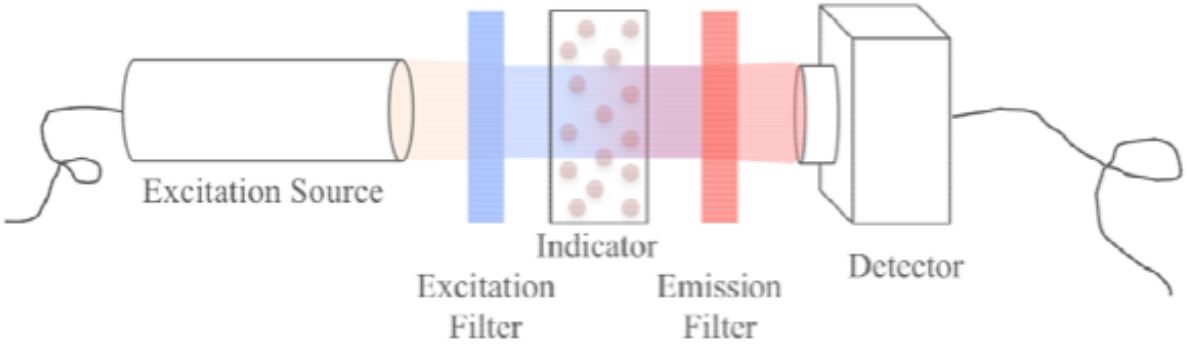


Figure 16. Simplified luminescent imaging system for oxygen sensing used for excitation of indicator material [2]

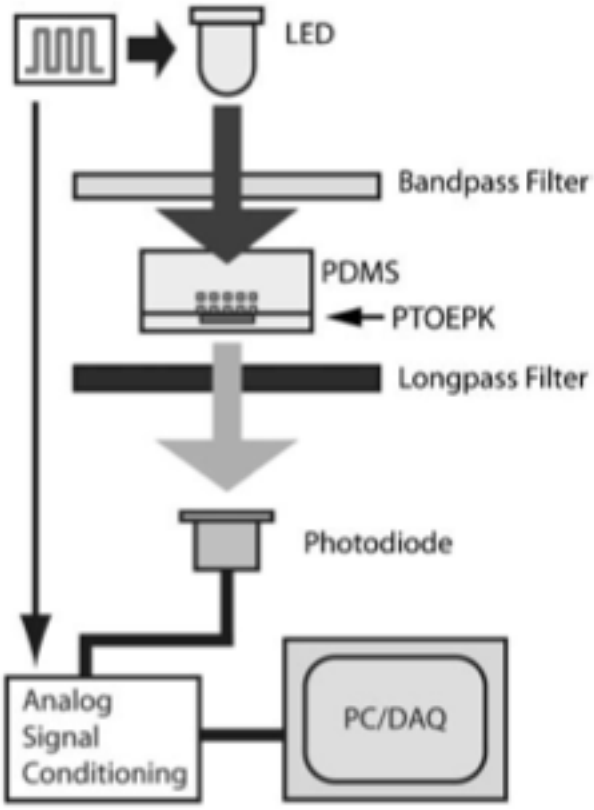


Figure 17. Optical and electrical components used in an excitation-detection system [3].

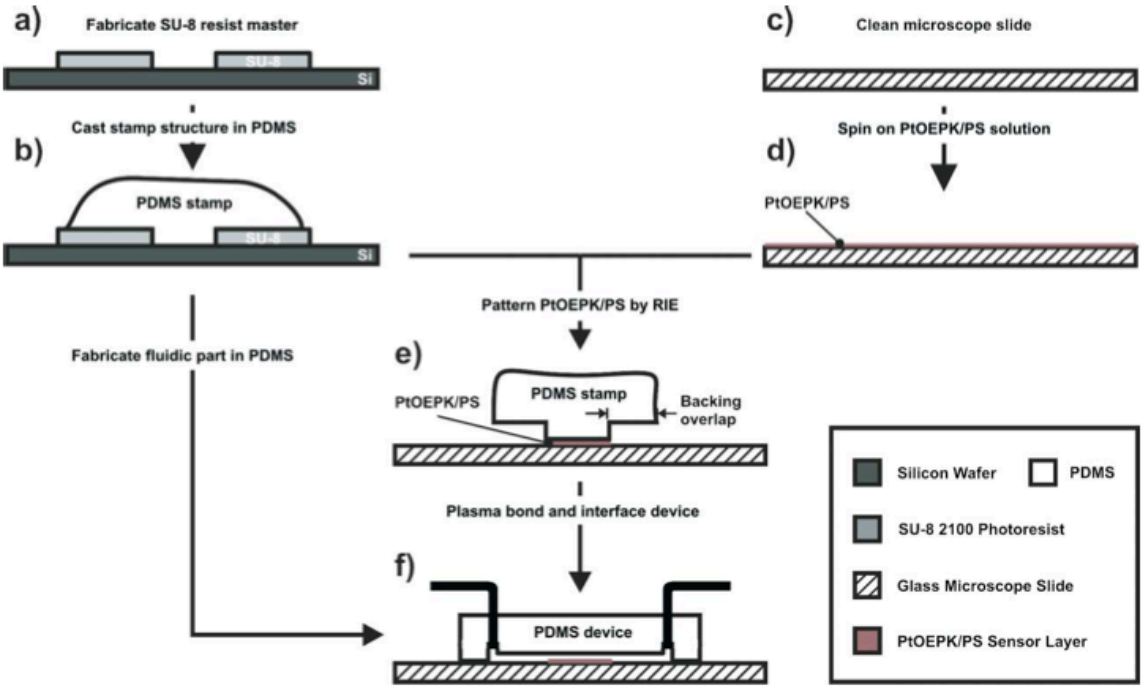


Figure 18. Schematic of the sensor fabrication process showing stamp fabrication in PDMS [4]

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