DEVELOPMENT OF AN OXYGEN SENSOR FOR CELL-BASED HYPOXIA STUDIES IN MICROFLUIDIC DEVICES

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ABSTRACT

Heart disease, the number one cause of death in the United States, has the potential to be combatted with stem cell therapy. In order to study the behavior of the stem cells under hypoxia, professor Ogle's lab desires a microfluidic cell culture device capable of developing an oxygen gradient and an oxygen sensor. A cell culture chamber device was fabricated by a previous design team. Our team has selected dichlorotris (1,10 – phenanthroline) ruthenium (II) hydrate (Rudpp) as an oxygen sensor molecule, designed a solid state sensor matrix, and tested the response of the sensor in different oxygen concentration. This sensor can be readily integrated with a variety of microfluidic devices.

INTRODUCTION

BACKGROUND

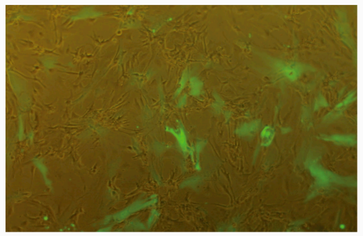


Figure 1: Green fluorescence shows fused adipose tissue derived stem cell with rat neonatal cardiomyocytes

Heart disease is the number one cause of death in the United states and demands new treatment options[1]. One proposed therapy is the use of stem cells. Research shows that stem cells can fuse with damaged cardiomyocytes in hypoxic conditions in order to regenerate damaged cardiac tissue, as illustrated in Figure 1.

PREVIOUS WORK

A BME design team in spring 2012 designed a PDMS microfluidic cell culture device, as pictured in Figure 2, for stem cell research[3]. Microfluidic technology is desirable for this application because the laminar flow phenomena in micro- environments can create an oxygen gradient.

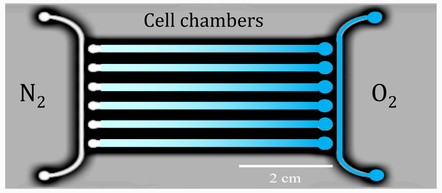


Figure 2: The design of the microfluidic device. Oxygen (blue) flows through the right-hand channel and diffuses to the left, creating a decreasing gradient. Nitrogen (white) flows through the left-hand channel .

COMPETING TECHNOLOGY

An oxygen sensor is required to monitor the concentration gradient across the channels of the microfluidic device. There are few sensors applicable to these devices. One such sensor is a platinum fluorophore based sensor; another is a thin film, ruthenium-based sensor. These sensor are often application specific and do not completely satisfy the gradient measurement requirements of this device[4,5].

PROBLEM STATEMENT

A real-time oxygen sensor capable of monitoring oxygen concentrations in a microfluidic device is required. The sensor should not inhibit or interfere with the stem cell behavior of interest.

DESIGN CRITERIA

- Non-cytotoxic
- Protocol can be completed in ten hours
- Compatible with existing device
- Accurate to within 2% concentration O_2
- Under 50 grams
- Optically compatible for microscopy

FINAL DESIGN

PROTOCOL

PDMS MATRIX

1. Prepare PDMS (1:10 ratio of curing agent to base); De-gas
2. Spin coat a layer of PDMS at 100 RPM on a silicon wafer. This produces a 561 µm layer
3. Bake PDMS for four hours; let it cool overnight
4. Remove and cut into desired dimensions
5. Soxhlet extraction at 90°C at 100% ethanol for 3.5 hours (4 cycles) to remove uncross-linked PDMS and increase adsorption

SENSOR INCORPORATION

1. Dissolve sensor molecule (Rudpp) in ethanol to make 50 µM solution
2. Submerge the PDMS matrix into sensor solution (Rudpp) and let it set overnight to allow full coating of the PDMS
3. Take the PDMS matrix out of the solution and attach to glass slide

SENSOR

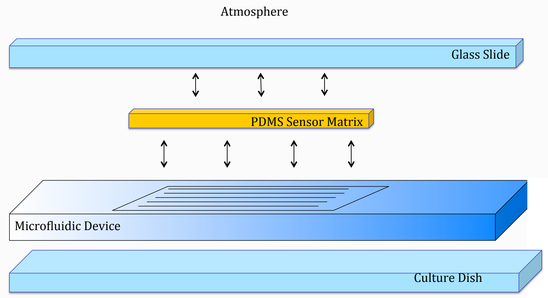
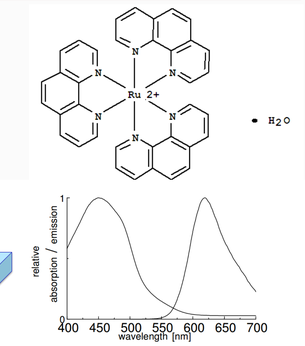


Figure 3: The final sensor design involves three distinct components: The first is a glass slide, which is used as an anchor for the sensor matrix. The glass slide prevents gas exchange between the channels of the culture device and the atmosphere. The sensor matrix is attached to the glass slide via adhesion and is composed of PDMS with a layer of Rudpp sensor molecule coated on top. The glass slide and sensor matrix are placed on top of the microfluidic device channels for oxygen concentration monitoring.



abs. λ_{max} 455 nm, luminescence λ_{max} 613 nm

Figure 4: The chemical structure and absorbance/ emission waveforms of the Rudpp sensor[6]

TESTING AND RESULTS

MATRIX MATERIAL

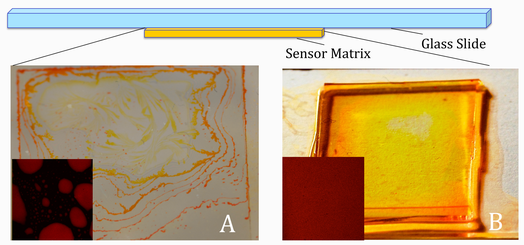


Figure 5: Image A is the Rudpp sensor incorporated (via soaking) onto a polystyrene matrix and Image B is the Rudpp sensor incorporated onto a PDMS matrix. The polystyrene matrix, after drying, shows non-uniform coating of the molecule with banding and beading. The PDMS matrix, after drying, has a uniform and smooth coating which are ideal properties for accurate oxygen sensing. High homogeneity is required in this project and the uniformity was confirmed in fluorescent microscopy.

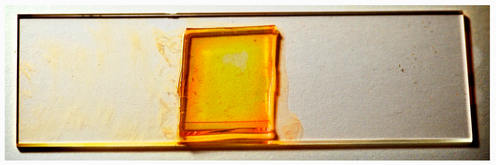
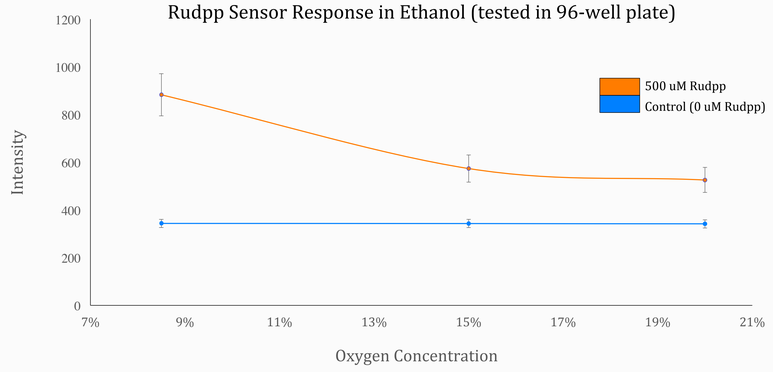
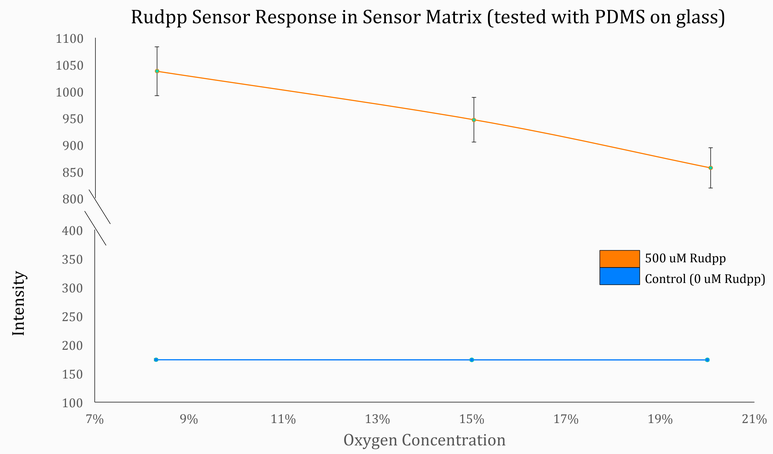


Figure 6: The testing of the matrix material resulted in a PDMS matrix ideal for a solid-state sensor. The sensor matrix was then placed onto a glass slide to confirm that it would adhere properly. The final sensor was placed on top of the microfluidic device channels to confirm the dimensions were appropriate.

Component	PDMS + Glass	Rudpp Sensor	Total
Cost	$2.00	$84.80 (per bottle of 1 g) / ~ $1.00 (per sensor)	$3.00 (per sensor)





FUTURE WORK

- Test the sensor with more concentrations of oxygen
- Test the response time of the sensor
- Test the microfluidic device to see if it can develop a gradient
- Test the microfluidic device with cells
- Test the microfluidic device with the sensor integrated
- Calibrate the device with the integrated sensor

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- Design Teams 2012
- BME Department

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