Generation of an Accessible and Versatile Hypoxia Chamber
Product Design Specifications
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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.