Microfluidic Gas Diffusion Platform

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Abstract

Myocardial infarctions, commonly known as heart attacks, are responsible for one in four American deaths each year. Ischemia is the lack of oxygen delivery, and is a cause of cardiac cell apoptosis. Heart cells are terminally differentiated, so in the event of mass apoptosis, the heart is severely weakened. Current cardiac research is focused on determining the ideal conditions for fusing mesenchymal stem cells with ischemic heart cells. Combining fine gas control and detection with a microfluidic system will simulate an in vivo environment more closely than previous methods. The end goal of this design is to develop a microfluidic-based hypoxia chamber to facilitate studies involving oxidative stress, ischemia, and reactive oxygen species (ROS)-mediated cellular pathways.

Background and Motivation

- Myocardial infarctions cause one in four American deaths each year [1]
- Cardiomyocytes die within 3-4 hours of oxygen deprivation [2]
  - Terminally differentiated (cannot divide)
  - Seek ideal conditions for differentiation and fusion of mesenchymal stem cells with heart cells
- Commercial hypoxia chamber (**Figure 1**):
  - Not cost effective [3]
  - Slow equilibration time [4]
  - Unable to establish spatial gradient
  - Inaccurate model of in vivo conditions
  - Limited to gas input

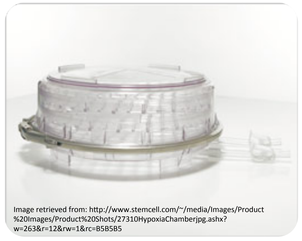

Image retrieved from: http://www.stemcell.com/~/media/Images/Product%20Images/Product%20Shots/27310HypoxiaChamberjpg.ashx?w=263&r=12&rw=1&rc=B5B5B5

Figure 1: Macro-scale hypoxia chamber

Design Criteria

- Develop microfluidic based hypoxia chamber
- Generate spatial O_2 gradient 21% - 0%
- Materials housing cells must be biocompatible
- Channel dimensions 250 – 750 µm x 250 – 500 µm (W x H)
- Function inside cell incubator at 37° C and 5% CO_2

Device Design

- Operates by diffusion of O_2 gas
- O_2 and N_2 gas inputs
- Variable gradient function of pressure input
- Eight longitudinal channels orthogonal to gas inputs (**Figure 2**)
  - Longitudinal channel dimensions = 31 x 0.75 x 0.25 mm (LxWxH)
  - Regions for cell culture

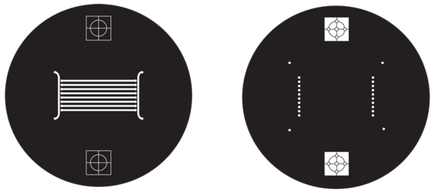

Figure 2: Photomasks for top and bottom layers of master template

Gradient Model

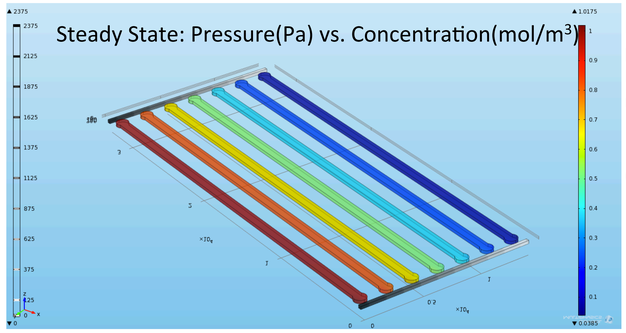

Figure 3: At input pressure of 2,500 Pa, O_2 gradient ranges from 1.26 [mol/m³] to 0.1 [mol/m³] (COMSOL®)

Navier-Stokes Incompressible Flow Equation

$$\rho\frac{\partial u}{\partial t}+\rho(u\bullet\nabla)u=\nabla\bullet[-pI+\mu(\nabla u+(\nabla u)^T)]+F$$

Diffusion/Convection

$$\nabla\bullet(-D_i\nabla c_i)+u\bullet\nabla c_i=R_i$$

Channel Concentration as a Function of channel # & Input Pressure

$$\frac{n[mol]}{V[m^3]}=\left[\frac{14{,}500-((channel\#)\bullet 1{,}803.57)}{14{,}500}\right]\bullet\frac{P[Pa]}{R\left[\frac{J}{K\bullet mol}\right]\bullet T[K]}$$

Device Fabrication

Figure 4: SU-8 Master Template

Master template production

- Spin SU-8 onto silicon wafer
- Pre-bake cycle (Intervals of 65° and 95° C)
- Expose photomask covered wafer to UV light to crosslink SU-8 (**Figure 4**)
- Post-bake cycle (Intervals of 65° and 95° C)
- Repeat for subsequent feature layers
- Rinse in SU-8 developing solution to remove non-solidified regions

Polydimethylsiloxane (PDMS) mold

- Pour PDMS onto master template
- Place in vacuum until void of bubbles
- Bake on hot plate at 80° C for 240 minutes
- Separate PDMS from silicon wafer

Oxygen Detection

- Methylene blue [$C_{16}H_{18}N_3SCl$] indicator reaction with dextrose [$C_6H_{12}O_6$] in basic solution [NaOH] (**Figure 5**)
  - Solution blue in presence of O_2 (Oxidized)
  - Solution colorless in low O_2 concentration (Reduced)

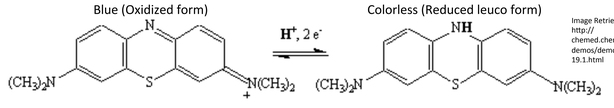

Image Retrieved from: http://chemed.chem.purdue.edu/demos/demosheets/19.1.html

Figure 5: Reversible oxidation – reduction reaction of methylene blue

Testing

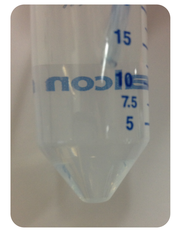

Figure 6: Methylene blue solution saturated with N_2 gas

Figure 7: Methylene blue solution saturated with 21% O_2 gas

Figure 8: Methylene blue solution saturated with 95% O_2 gas

- 3 replicates tested at each of the following: 0%, 21%, 95% O_2 concentration for color channel intensity (**Figures 6, 7, 8**)
- Color intensity calculated for aggreage and indiviual color channels using Image J
  - Sample size of 500 x 600 pixels
- Results statistically significant for all channels (**Figure 9, Table 1**)
  - Green→Ideal color channel for analysis

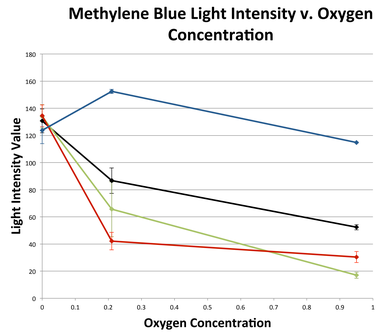

Table 1: Single Factor ANOVA Results

Light Intensity Measurement	P-Value
RGB	0.0000444*
Green	0.0000909*
Red	0.00000195*
Blue	0.000513*

*p<0.001

Figure 9: Methylene blue intensity with corresponding O_2 concentrations and standard deviation

Future Work

- Channel redesign goals:
  - Reduce gradient formation time
  - Create larger connection features
- Implement ruthenium (fluorescence) based O_2 sensor strips
- Inhibit cross channel diffusion by coating channel interior with paraline
- Culture stem cells within the device

Acknowledgements

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[1] Centers of Disease Control and Prevention. 2012. Heart Disease Facts and Statistics. http://www.cdc.gov/heartdisease/statistics.htm.
[2] Chao, Wei, Yan Shen, Ling Li, and Anthony Rosenzweig. "Importance of FADD Signaling in Serum Deprivation - and Hypoxia Induced Cardiomyocyte Apoptosis." *The Journal Of Biological Chemistry* 277.35 (2002): 31639-31645.
[3] "Price List." *Billups-Rothenberg, Inc.* N.p., 2001. Web. 2 May 2012. http://www.bricubator.com/pricelist.htm
[4] Allen, Corrie, Kelly Schneider, and Carl White. "Limitations to oxygen diffusion and equilibration in in vitro cell exposure systems in hyperoxia and hypoxia." *American Journal of Physiology* 281 (2001): 1021-1027.