Design of a Universal Microscope Incubator for Drug Screening of 3D Models of Engineered Myocardium

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***Abstract*— Challenges facing the production of new pharmaceuticals include the costs associated with recalls from adverse effects and a lack of effective, low-cost model systems for predicting them. Three-dimensional (3D) tissue models offer assays that facilitate faster, more robust pre-clinical screening for therapeutics. These assays require handling multi-sample drug perfusion at cell-viable conditions for long term testing and imaging experiments. Current devices do not achieve these criteria in a cost-effective manner. The goal of this project is to design and develop a microscope incubator system for imaging and testing of 3D tissue constructs in drug screening. Tissue engineered myocardial constructs will be used for proof-of-concept. Initial findings suggest our device presents a viable diagnostic tool using 3D tissue constructs for therapeutic screening.**

Keywords—tissue engineered myocardium, drug testing, incubator

I. Introduction

Introducing a new drug to the market is inefficient, and can inflate to around $10 billion [1]. Common complications that occur in the production of therapeutic compounds arise from adverse effects undetected through conventional testing. To detect adverse effects, about an extra $10 billion must be spent due to the lack of adequate cost-effective *in-vitro* testing methods available [2].

Recent studies have focused on the development of 3-Dimensional (3D) *in-vitro* tissue models in place of conventional 2-Dimensional (2D) testing [3]. Particularly for cardiovascular models, 3D-cultured cardiomyocytes show characteristics similar to those *in-vivo* [4][5]. The mechanical and electrical characteristics of these 3-D models can be monitored for real-time analysis and evaluation of drug performance. However, during imaging cells are present in an environment unsuitable to maintain homeostasis, making this method unsustainable for long-term monitoring. A device that sustains long-term physiologic conditions for benchtop analysis of these 3-D scaffolds would enable researchers to accurately examine the temporal behavior of cells when exposed to new therapeutic molecules.

Such a device would need drug perfusion capabilities and must allow for real-time viewing of samples using fluorescent microscopes. The samples must also be maintained at physiologic conditions to maintain proper cell function. In this study we aimed to develop an incubator system that would allow for imaging of 3D engineered tissues for pre-clinical drug screening. We anticipate this work will reduce the cost of drug screening substantially by providing a more accurate diagnostic tool for detecting adverse effects of therapeutic compounds.

II. Methods

A. Device Design

The device we developed is based on a re-usable 6-well cell culture plate cover with perfusion inputs and a disposable plate bottom (Figure 1). Data from a thermistor and CO_2 sensor regulate device conditions via an Arduino board interface (not shown).

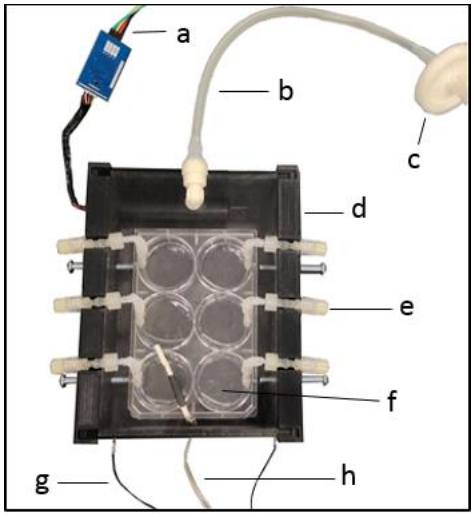

Figure 1*: Device design: a) CO_2 sensor interface, b) CO_2 input tubing, c) CO_2 in-line filter, d) device holder, e) perfusion inputs, f) modified 6-well plate cover, g) transparent heater (under device) wires, and h) thermistor wire.*

B. Temperature Control

An indium tin oxide (ITO) transparent heater (Thin Film Devices©, 6”x4”) was used below the cell culture plate to maintain a temperature range of 35-37°C. The distribution of heat was observed using infrared imaging. A control system was developed using a thermistor in a device well and an Arduino© board interface to modulate the temperature. All wells

were filled with 3 mL of water and the system was run for 30 minutes. A control 6-well plate was filled with room temperature water and was left unheated on the benchtop. The results were recorded using thermocouples. The effect of the transparent heater on imaging quality was verified by comparing the fluorescence intensity of fluorescently tagged samples.

C. CO_2 Control

To allow for benchtop regulation of CO_2 in the device, a 1-liter CO_2 tank and regulator (Ista©, Product #AGU00677) was used. Power for the tank regulator was controlled through an Arduino© board using data from a CO_2 sensor inside the device. The sensor chosen was a Non-Dispersive Infrared (NDIR) CO_2 sensor (Sandbox Electronics©, SKU# SEN-000030) that could read and regulate internal CO_2 levels within a range of 4-6%. A calibration curve for the sensor was obtained using a cell culture incubator set to different levels of CO_2.

D. Perfusion Testing

Perfusion into the wells was achieved using 0.86mm inner diameter (ID) polyethylene tubing led through the re-usable cover of the 6-well plate. The tubing was attached to connectors and a needle injection Luer lock to maintain sterility during perfusion via a syringe on the benchtop. Perfusion was observed through time-lapse photography by injecting food dye into wells filled with 3 mL of water.

E. Humidity Control

Humidity in the cell culture plate was created by filling the basins between the culture wells with 3 mL of sterile water. A control plate with water was left unheated on benchtop. Humidity at 37°C was measured using a LoggerPro® humidity sensor and system to ensure a humidity range between 95-100% relative humidity.

F. Cell Culture

Cell culture of C2C12 cells on fibrin constructs was conducted according to a protocol described previously [6]. Perfusion was assessed by staining with LIVE/DEAD® assay (ThermoFisher©) via the perfusion system for experimental plates. The C2C12-seeded constructs were imaged through fluorescent microscopy and analyzed using ImageJ software to obtain cell counts.

III. Preliminary Results

Distribution of temperature imaged with infrared in the water-filled 6-well plate was within the desired temperature range. Temperature recorded in the wells maintained 35-37°C during the 30-minute test (Figure 2). For CO_2 regulation, the control system maintained internal CO_2 between 4-6% (40,000-60,000 ppm) after steady state was reached (Figure 3).

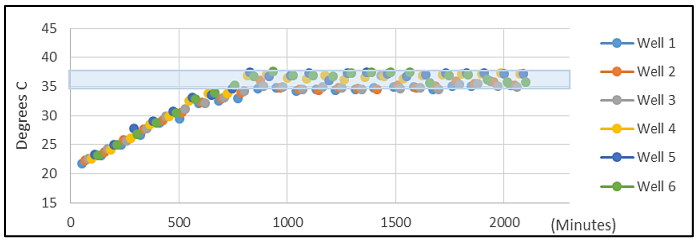

***Figure 2**: Thermocouple reading of good temperature.*

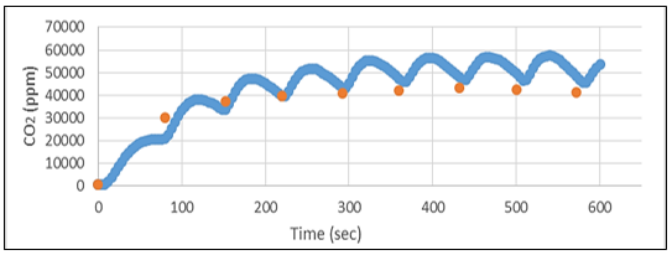

***Figure 3**: CO2 sensor measurements (blue) and Vernier sensor (orange) with control system over time, measured in parts per million (ppm)*

Perfusion was validated by observing uniform dye distribution into the wells, and by the LIVE/DEAD® stain in C2C12 constructs. Cell counts were found to be comparable with control (Live: 836 ± 163, Dead: 64 ± 23) and experimental (Live: 934 ± 178, Dead: 39 ±5). The similarity in cell counts indicate that a solution perfused through our system is dispersed evenly throughout the construct. Finally, the humidity in each well maintained 90-100% relative humidity over 7 hours with our temperature system, as measured by the Vernier humidity probe. In comparison, the control plate maintained a range of 25-35% relative humidity.

IV. Conclusions

These data suggest that our device maintains the necessary conditions for culturing 3D tissue constructs for benchtop pre-clinical drug testing. In the future, our device will be tested for longer time periods using cardiomyocyte-seeded constructs. Ultimately, we envision use of this system with the latest innovations in tissue engineering to reduce the cost and time associated with pre-clinical drug testing.

V. References

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