Design and Creation of a Perfusion Circuit for Advanced Long-term Neuromodulation

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Introduction

Context: The need for long-term *in vitro* neurophysiological evaluation tools has become increasingly critical to sustain advances in the neurotechnology and neuroscience [1,2]. The main challenge to such systems resides in the maintenance of neuronal cells outside incubator conditions over 3-4hours. Several engineering solutions have been proposed which require adaptation to the applications targeted [3,4].
Innovation: Here, we present a novel perfusion system for long-term (weeks) *in vitro* neuromodulation. The construct incorporates an adaptable media supply system, a gas exchange device, a temperature controller, a filtering system and a cell hosting compartment integrated with a reader-stimulator for electrophysiology and neuromodulation.
Applications: This current technology has the potential to enable significant neurotechnology currently limited by the duration of neuronal cell sustenance outside the incubator and can open doors to high impact applications such as *in vitro* long-term drug testing, disease modelling and research into how neural systems functions.

Perfusion Circuit Design

Gas Exchange System

Used to diffuse gas into the media to maintain pH and dissolved oxygen of media.

Gas Supply

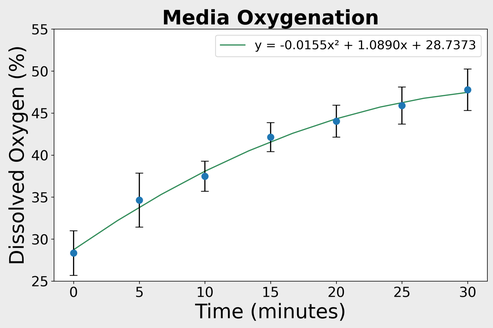


Figure 1. Oxygenation of media using gas exchange system

The gas exchange system presented efficiency in supplying oxygen to the cell culture media. Using this system, an increase in Dissolved Oxygen (DO2) from 28.3% to 47.7% was effected within 30 min under experimental conditions.

Heating Plate

Used to control the media and cell culture temperature.

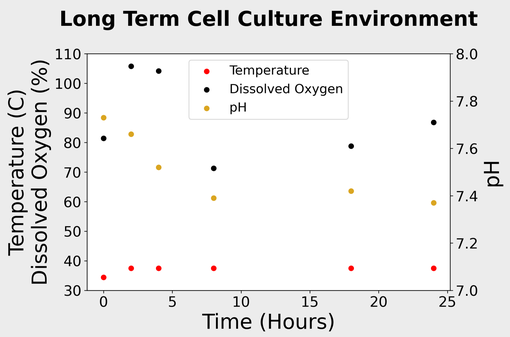


Figure 2. Temperature, dissolved oxygen and pH maintenance over 24 hours in perfusion system.

The closed-loop control system maintains the cell culture environment at 37.5 degrees, pH 7.4 and dissolved oxygen above 70% over long periods of time.

MEA Cap

Enclose the cell culture within the perfusion circuit including an integrated bubble control.

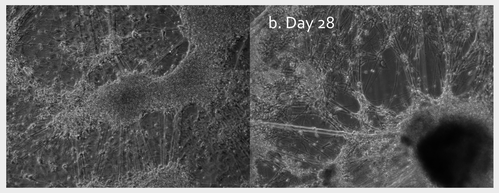
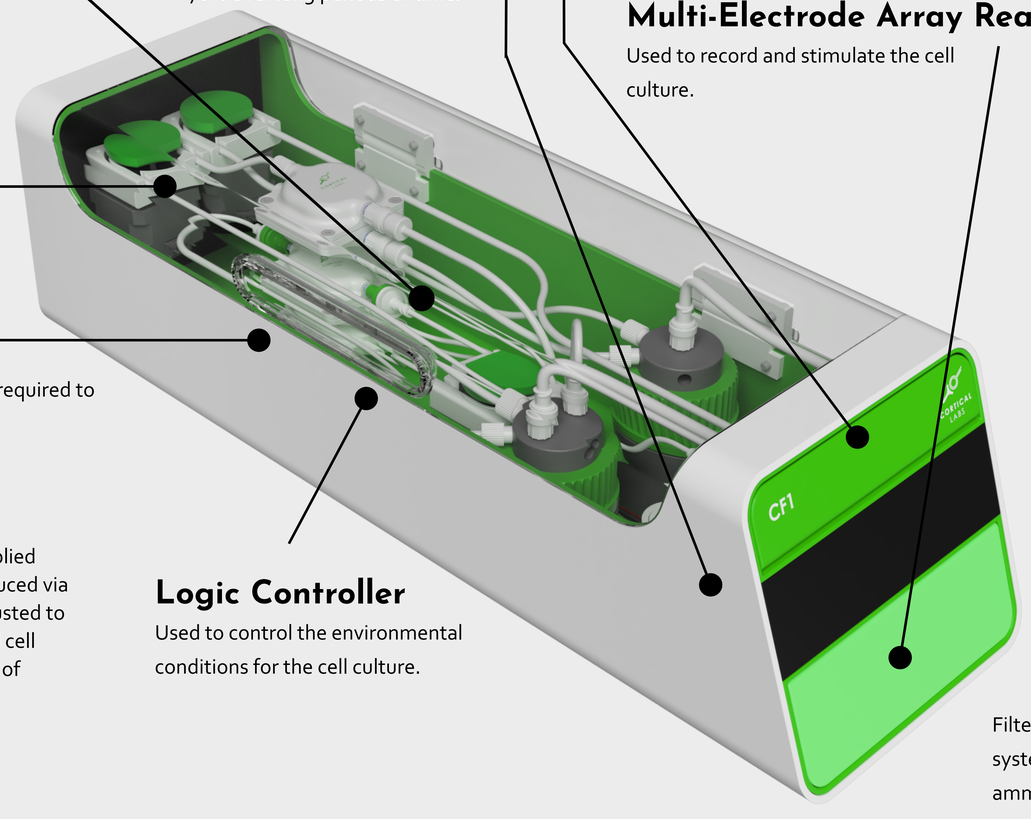


Figure 4. Dual SMAD Inhibition differentiated neurons at a. Day 0 and b. Day 28. Cell and neurite morphology were maintained over 4 weeks.

Dual SMAD inhibition differentiated neurons were hosted in the perfusion system (37^{O}C; 5% CO2). Cell survival and neural cell morphology (neurites) were maintained over 28 days.

Multi-Electrode Array Reader

Used to record and stimulate the cell culture.



Peristaltic Pump

Used to control fresh media flow, waste media flow and gas flow rates within the system.

Gas Mixing Chamber

Used to mix CO2 and O2 and control the concentration levels required to control the dissolved oxygen and pH within the media.

Media Supply

Media of different composition (low/ high protein) can be supplied through the perfusion system. Risk of bubble formation is reduced via bubble guides present in the MEA cap. Supply rate can be adjusted to the cell metabolic needs and to prevent mechanical stress and cell detachment (0-7000 ul/min). Using this system, the frequency of media change was reduced to once every 2 weeks (40ml).

Logic Controller

Used to control the environmental conditions for the cell culture.

Long Term Recording and Stimulation Pong Gameplay

Live feed of perfusion circuit with MaxWell MEA reader, media reservoir and peristalic pump in view.
Raster view displaying 90 seconds of neuron activity and stimulation used within the Pong game environment. The neurons are embodied within the closed-loop Pong game environment in a structured method, where the neuron activity is used to control the paddle and the ball position is stimulated to the neurons.
Based on this implementation, learning has been evidenced within five minutes of real-time gameplay [2]. The neurons, while housed in the perfusion circuit, were able to survive and 'play' the game Pong over a period of 18 hours.

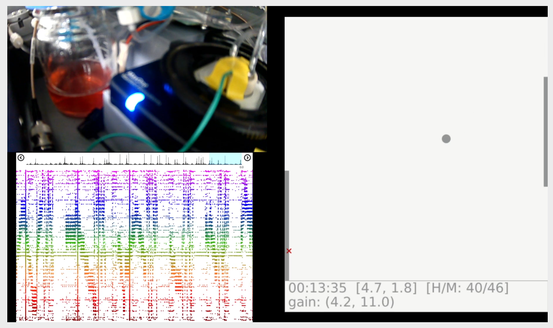


Figure 5. Pong gameplay within the perfusion system. (Neuromodulation using MaxWell MaxOne MEA Reader)

Filtration System

Used to filtrate the media of waste while keeping protein and growth factors within the system.

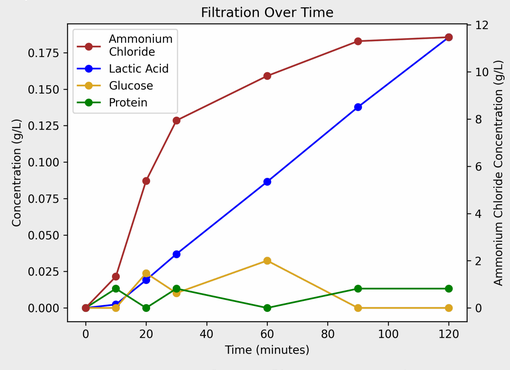


Figure 3. Perfusion filtering over 2 hours.

Filtering of lactic acid and ammonium chloride (cell culture waste) was tested in the filtration system and was quantified to 0.0024g/L/min for lactic acid and 0.0958g/L/min for ammonium cholride while keeping in protein and glucose under experimental conditions.

Outlook and Applications

The perfusion system has unique features to maintain neuronal cells in vitro for several weeks outside incubator conditions which can enable long-term MEA and other electrophysiological recording and neuromodulation of neuronal cells in vitro over weeks and potentially months. The perfusion technology can facilitate high-impact applications such as long-term advanced drug-testing, in vitro neuronal disease modelling, which can be combined with research into the fundementals of neural computation.

Acknowledgements: Dept. Neuroscience - Monash University (The Alfred Centre, Melbourne) and Dr. Bradyn Parker.

References

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