Development of Microscope Stage Incubator for Long-term Monitoring of Neuronal cell Culture

Kailash Karthikeyan, Shruti Kanakia, Sreevidhya Banda, Digant Davè, and Young-tae Kim
Neuro-Optical Engineering Laboratory
Department of Bioengineering, University of Texas at Arlington, Arlington, TX

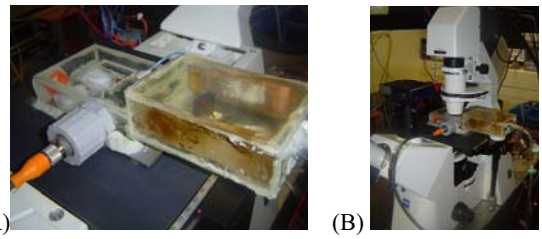
Introduction

The desirability to study the cells continuously in relation to their constant development has been long desired. The present technology does not have provision for continuous observation of neurons to provide a better insight on their characteristics and changes under different conditions. Neurons responses to external stimuli are robust and spontaneous and this arises need to constantly monitor them after the external stimulus is applied for understanding their reaction on exposure to the stimuli. The use of microfluidic chips also help in the isolation of individual axons making it possible to understand the characteristics and responses of an individual axon. The commercial microscopic stage incubators help in the culturing and observing the cell but however are not customized to our needs[1]. A custom made incubator in required to observe the cells continuously over time. The customized incubator is made with a temperature controller system, CO_2 and air control system to maintain the optimum conditions.

Materials and Methods

Fabrication of microscope stage incubator

The fabrication of the microscope stage incubator was done in three steps. The first step was the design of the incubator. The incubator consists of an upper plastic chamber and a lower metal stage insert. The upper chamber maintain sterilized and adequate environment for neuronal cell culture. The chambers were fabricated using anti-fog clear cast acrylic sheet to minimize a condensation. The chamber also contained two separate filtered gas inlets (air and CO_2 gas) and a filtered gas outlet. The center of the chamber had an optical window which was covered with coverslip to allow microscopic observation (Figure 1).



(A) (B)

Figure 1. (A) shows the two chambers wherein one serves as the chamber for mixing gases and in the other the neuronal cells are cultured. Figure (B) shows the entire apparatus setup with the microscopic incubator on the microscope

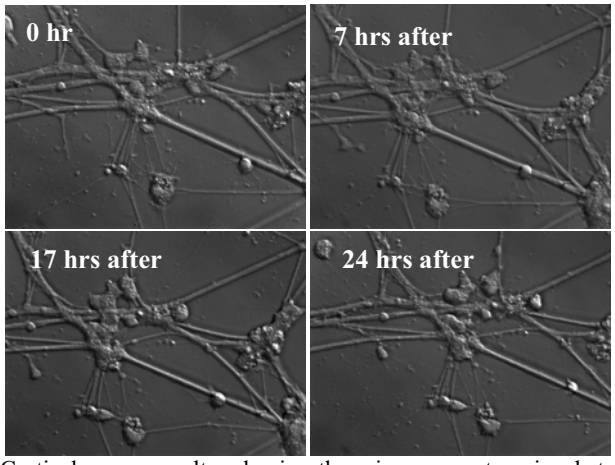
The second part is an automatic temperature controlling where the temperature is automatically regulated at preset level using silicone rubber heaters and a temperature regulator. Two silicone rubber heaters were attached on the side wall of the upper chamber. Thin copper foil was placed on the heaters using a thermal adhesive to act as a heat sinker and minimize contamination. The measured temperature was feedback to the regulator via thermocouple and the regulator controlled the heaters. The heat is circulated by using a fan.

The third part of the incubator is the CO_2 and air controller to maintain appropriate CO_2 level (5% CO_2 and 95% air) at the neuronal culture chamber using automatic feedback circuits. The percentage of CO_2 was continuously measured by CO_2 sensor and sent to a feedback circuit (simple comparative IC circuit). If the percentage of CO_2 level was lower than reference value, it opened a solenoid valve to allow CO_2 gas into the incubator. When it was over the reference value, it closed the solenoid valve. Needle valve was utilized for fine adjustment. Air was automatically fed every two hours for 3-5 min using a timer circuit. Both CO_2 and air were filtered via sterilized syringe filter and forcefully mixed by a fan.

Cortical neuronal culture on microscope stage incubator

Cortical neurons were isolated from embryonic 16-18 day rat pups. The cortical tissues were dissected, cleaned, and enzymatically dissociated (0.25% trypsin) for 20 minutes. The cortical tissues were triturated and the resulting cell suspension was loaded into the microfluidic devices. The devices consisted of two compartments (100 μm height) bridged by multiple microchannels (15 μm width, 7 μm height, and 2 mm long). After 15 minutes of incubation at 37°C, culture medium (Neurobasal medium supplemented with B-27) with growth factors (BDNF and NT-3, 10ng/ml) was slowly added into the device. The medium with growth factors was changed every two day. The microfluidic device containing cortical neurons was placed on the center of the incubator and covered with the chamber under sterilized environment. Two humility containers (wet sterilized cotton gauze) were placed next to the device. The chamber was then sealed with a removable sealant and placed on the inverted microscope. The 40x objective was used to continuously monitor the neuronal activities for 24 hours. The appropriate temperature and gas was automatically controlled by each feedback circuit.

Results and Discussion



Cortical neurons cultured using the microscope stage incubator were healthy and no degeneration-related fragmented axons were observed. Also, a neuronal network was well maintained. CO_2 was maintained at the level of 4.75% to 5.05%. Air was fed every two hours. The temperature was maintained between the ranges 36.6 to 37.2°C. This temperature was reached in a time range of 3-4 minutes. In conclusion, we have demonstrated that the custom built microscope stage incubator allows one to long-term monitor neuronal activities under microscope. The incubator can be utilized as a comprehensive tool to optically observe cellular and molecular response to various endogenous and exogenous stimuli.

References

1. J. Thos. Patterson. A Microscope-Stage Incubator, 324-327(1908)
2. Cold Spring Harb. Protoc.doi:10.1101/pdb.prot4792 (2007)