Deliverable 3.2. – Demonstrator: Construction of the Physiological Chamber

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1 Version History

Version	Summary of Change	Written by	Approver	Date
0.01		Pablo Loza-Alvares	Edik Rafailov	Jan 2018
1.0	Approval by Coordinator	Derek McKenzie	Edik Rafailov	19th Feb 2018

2 Scope

The scope of Deliverable D3.2 is the demonstration of the construction of the physiological chamber. The chamber will be designed to maintain the correct physiological conditions for the neurons to remain alive during the imaging sessions and it will accommodate the scaffolds in a stable position compatible with the optics required for high resolution light-sheet imaging.

3 Introduction

For measuring both the structure and calcium activity of iPSC derived neuronal cell cultures, the cells should be maintained in good physiological conditions during the whole imaging experiments. In the following, we describe the construction of a physiological chamber for the aforementioned purposes, and following the design proposed in D3.1.

4 Main Body

4.1 Construction of the physio-logical chamber/incubator

According to the previous design of the physiological chamber/incubator for MESOBRAIN, see Report for D3.1, in the following we describe the construction and characteristics of its main components:

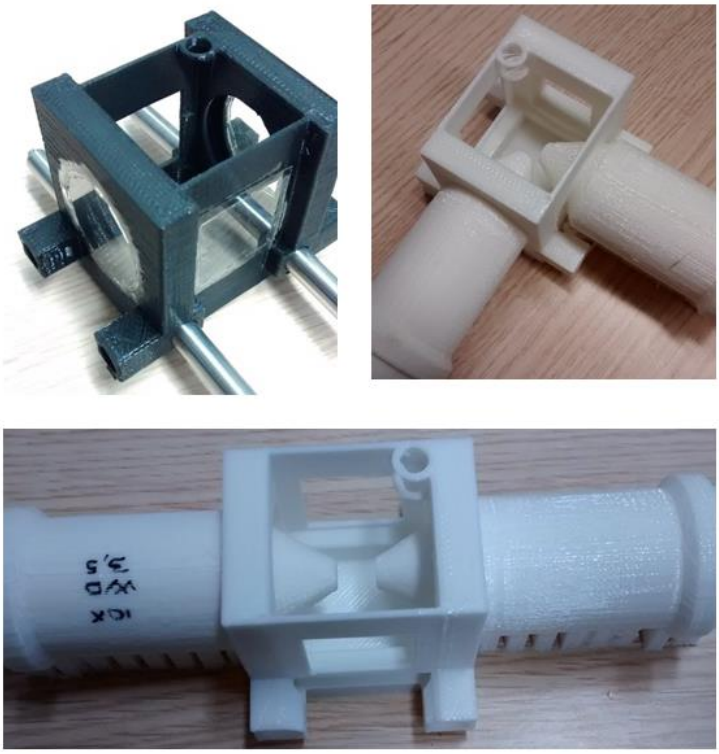

Figure 1. Different chamber core designs made with a 3D printer.

Chamber's core

Thanks to the 3D printing technologies available at ICFO, we can fabricate a number of chamber designs that can be then tested. In such sense, we have designed and printed several chamber models that mimic several imaging scenarios that we can encounter during the project. Figure 1 show three kind of chambers: i) a chamber optically accessible through all 4 orthogonal directions and in which LSFM is achieved using external (air) objectives, ii) a chamber for LSFM with both excitation and detection using immersion–type objectives, and, iii) an immersion-type chamber for dual detection and dual external excitation. All of them can hold approximately the same water volume of 10ml. Besides, all the required attachment structures for the temperature sensor, heating pad and perfusion in/outlets where taking into account in the designs.

Figure 2. Temperature controller components.

Temperature control

A custom-made temperature controller box was built for setting and stabilizing the temperature in the incubator chamber. This was assembled with the following parts:

- Temperature Controller: OMRON E5CN-H with output voltage capabilities of 230V and 12V, 3A maximum current and integrated PID control.
- Temperature sensor: PT100 waterproof sensor
- Thermometer: model WVR-620-1886. Used to measure the temperature at the sample location.
- Heater: Thermistor 12 Ω, 25W (connection to the 12V controller output)

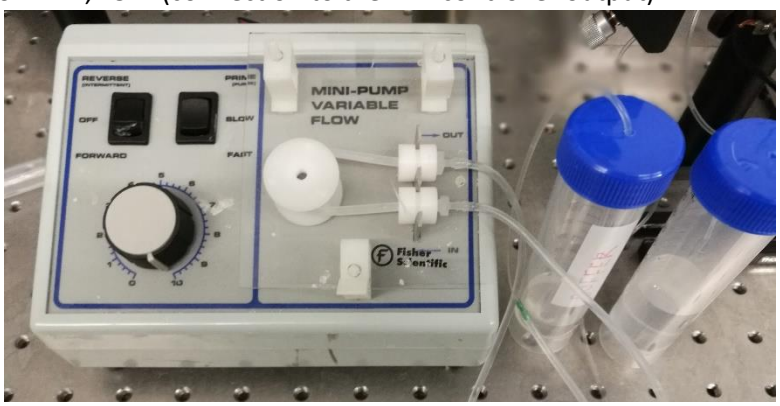

Figure 3. Perfusion system.

Perfusion system

The perfusion of the cell culture media is realized through the inlet and the outlet of the chamber's core. The chamber is connected to a perfusion pump, to the media supply bottle and to the media

waste bottle, using Tygon tubing. We have available 2 perfusion pumps to cover the different flow rates required for the imaging experiments:

- Bioptechs pump: 0.8-30 µl/min (ultra-low flow rate)
- Fisherbrand mini-pump: 50-500 µl/min (low-medium flow rate)

An in-house CO_2 tank is available to control the pH of the media by bubbling in a dedicated flask. One of the pumps connected to the supply/waste reservoirs is shown in Figure 3.

4.2 Characterization of the temperature control on the chamber

Figure 4. Arrangement for temperature stability tests: (left) Detail of the temperature sensor and the heating pad attached to the chamber and (right) Detail of the temperature measuring points, one on the corner and another in the centre of the chamber.

Figure 4 shows the setup employed for monitor and control the temperature of the water contained in the chamber. The important parameters are the long term stability, and the set-point reaching time. The initial settings of the experiments are the following:

- Open chamber configuration
- Initial volume: 30ml ultrapure water
- Room temperature: 25.6 °C
- Water initial temperature: 23.5 °C
- Set-point: 38 °C , PID settings: controller's default
- Total duration of the experiment: 21 hours.
- Two temperature measurements: corner and centre of the chamber

The results are shown in the curves of Figure 5. The temperature after one-hour stabilization keeps at an average temperature of 37.98 °C with a standard deviation of 0.23 °C (0.6% relative variation). The set-point of 38° was reached after 15 minutes, although the stabilization of the "hunting" oscillations extend over the first hour. Besides, we have found a time-averaged differential temperature between the corner and the center of the chamber of 0.6°. This difference implies that the set-point needs to be biased-corrected, as the sample will be located near the centre of the chamber, while the controller will read the temperature at the corner.
Another important measurement we did was the evaporation rate on the chamber. For the open top configuration we employed, we have found an evaporation rate of about 11 µl/minute. This can be compensated for using our perfusion system. Nevertheless, we expect to do most of the long-

term experiments in a closed top configuration where the evaporation rates will considerably decrease to a maximum of 1 µl/minute, which will not require much from the perfusion system for correction.

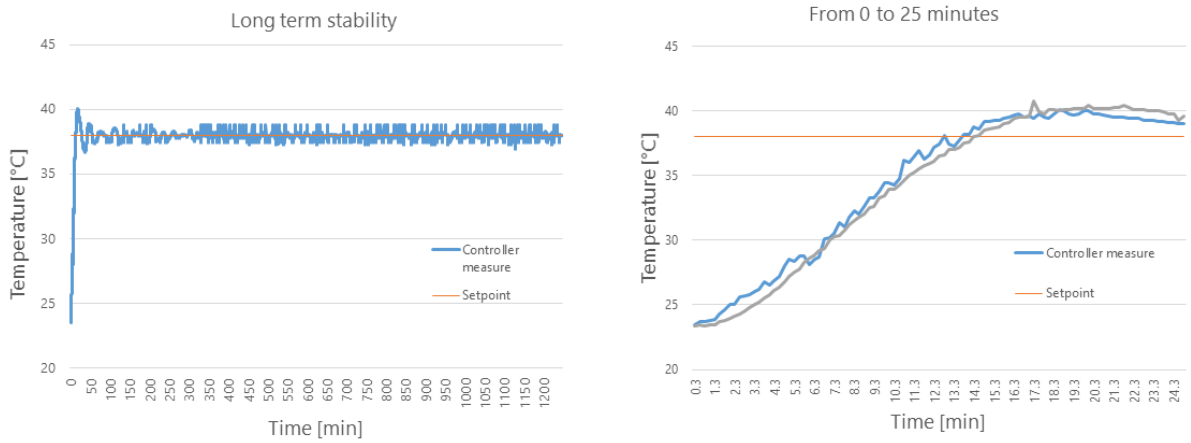

Figure 5. Temperature control on the chamber. Long term stability (left) and set-point reaching time (right) curves.

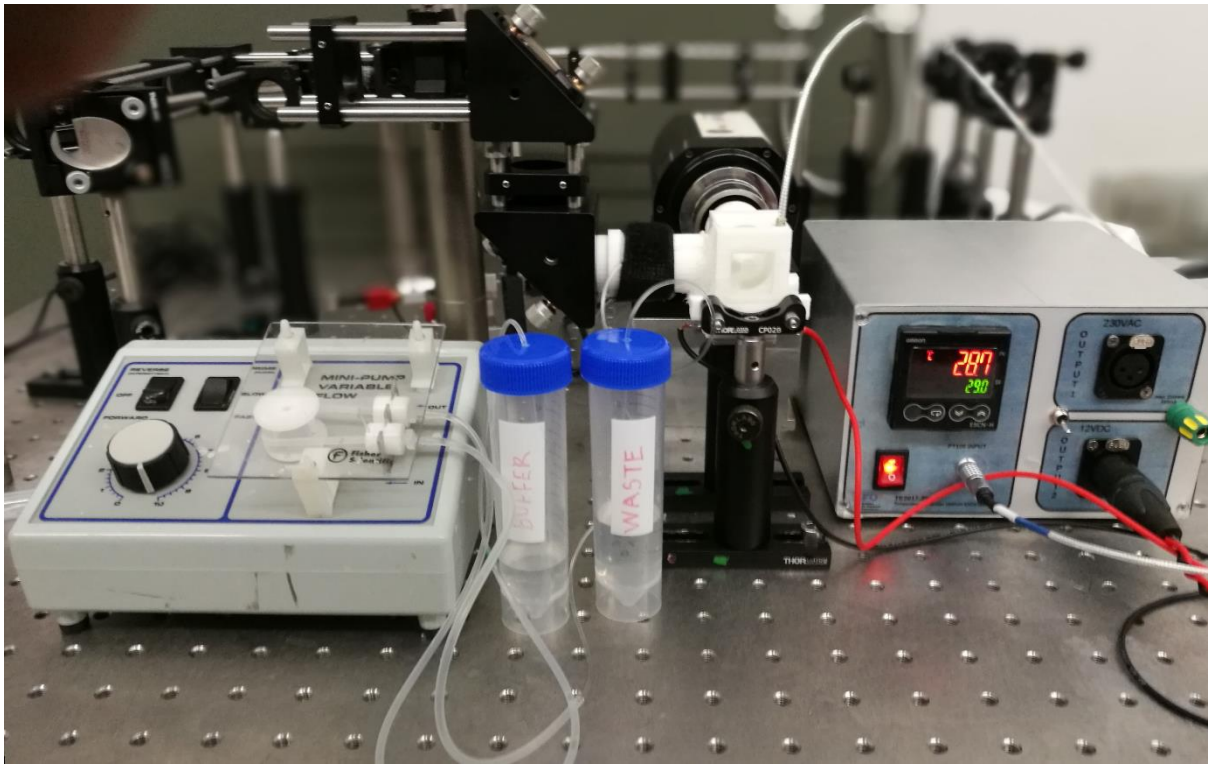

Figure 6: Full setup of the physiological chamber demonstrator, including the temperature control and the perfusion system (link to the media file physiological chamber).

4.3 Final set-up of the controlled imaging chamber

We have set-up a full demonstrator of the constructed physiological chamber that incorporates the temperature controller and the perfusion system in a realistic 3D printed version of the LSFM imaging system, which is shown in Figure 5 (and the associated multimedia file). We have verified that both the temperature and the buffer levels can be maintained at stable for long term imaging purposes.

5 Conclusion

A custom-made highly versatile physiological chamber has been constructed for long term imaging of 3D neuron cultures in a LSFM configuration. All the important parameters for cell survival and control, can be controlled for the different imaging experiments. The use of 3D printed technologies permits adapting the physiological chamber for a variety of different imaging experiments, even in a session basis manner. Once we have the 3D cell cultures available in the project, we will do the final optimization of the chamber including relevant biological parameters.

link to the media file physiological chamber

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