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Development of a microfluidic biochip for chronic monitoring of 3D neural tissues derived from human embryonic stem cells

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Abstract

The precise microenvironments required for optimal expansion or differentiation of stem cells are only beginning to emerge now, and the controlled differentiation of embryonic stem cells based on tissue engineering remains a relatively unexplored field. We have developed a small-volume *in vitro* system in which 3D neural tissues derived from embryonic stem cells are placed within up to four micro-chambers connected by micro-channels. Multi-electrode arrays (M.E.A.) were designed onto the porous membranes to record and stimulate electrophysiological activities from 3D neural tissues. A dedicated perfusion system based on air pressure was used to allow the circulation of the culture medium to the different micro-organs through a microfluidic system. This human biochip will enable the determination of toxicological profiles of new drug candidates.

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Keywords: Stem Cells; Microfluidic; Tissue Engineering; Multi-Electrode Arrays; 3D cultures.

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1. Introduction

"Cell biochips" containing engineered tissue interconnected by a microfluidic network, allows the control of microfluidic flows for dynamic cultures, by continuous feeding of nutrients to cultured cells and waste removal. Thus, these types of systems can enhance functionality of cells by mimicking the tissue architecture complexities when compared to in vitro analysis but at the same time present a more rapid and simple process when compared to in vivo testing procedures [1,2].

Embryonic or adult stem cells have demonstrated the potential to self-renew and differentiate into a wide range of tissues including neurons, hepatocytes, cardiomyocytes, and cells of the intestinal lineage, depending on the culture conditions [3,4,5,6]. The precise microenvironments required for optimal expansion or differentiation of stem cells are only beginning to emerge now, and the controlled differentiation of embryonic stem cells based on tissue engineering remains a relatively unexplored field.

2. Materials and Methods

We have developed a small-volume *in vitro* system in which intestine-like cells, hepatocyte cells and 3D micro-organs derived from embryonic stem cells.

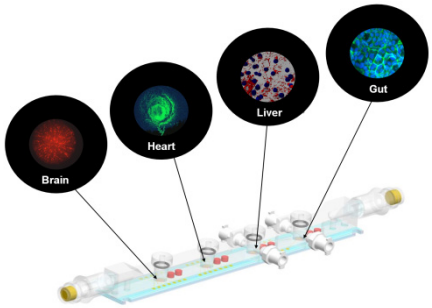

Fig. 1. Scheme of the multi-organs biochip where four different tissue-like structures are represented by microphotography.

Cardiomyocytes and neural cells were cultivated in four separate porous membrane microchambers connected by microchannels with the presence of biosensors at the different levels.

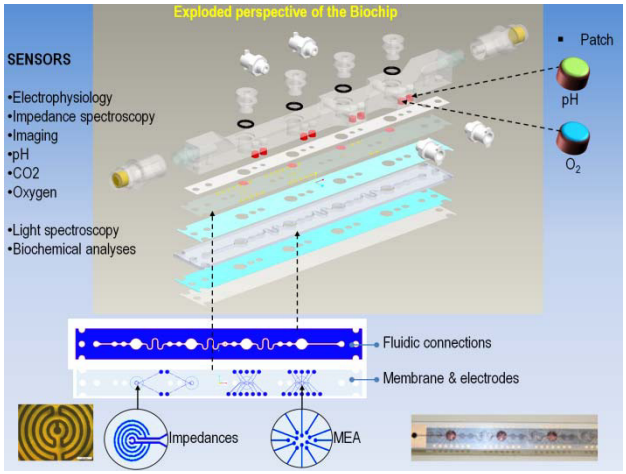

Fig. 2. Exploded perspective view of the Biochip showing the microfluidic connections as well as the different sensors placed along the system.

In a first step, we have used standard commercial membranes to obtain porous microchannel walls to supply nutrients and gases to cells cultured in 3D. Two types of electrodes were designed onto the porous membranes. Inter-digitized impedance electrodes were implemented to the first compartment to assess the tightness of the intestine epithelial cells. Two multi-electrode arrays (M.E.A.) were designed onto the porous membranes to record and stimulate electrophysiological activities from cardiomyocytes and 3D neural tissues.

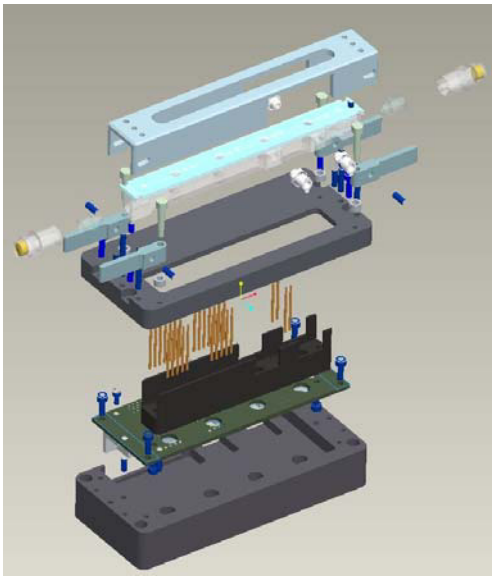

Fig. 3. Exploded perspective view of the electrophysiological connecting system.

A dedicated perfusion system based on air pressure was used to allow the circulation of the culture medium to the different micro-organs through a microfluidic system.

3. Results

In a first series of experiments, we could record simultaneously action potentials from beating clusters of cardiomyocytes as well as spontaneous activities of neural 3D tissues both being derived from human embryonic stem cells using the same culture medium.

We could also assess the functionality of intestine-like barriers using CaCo2 cells. Finally, hepatocyte cells (HepG2) grown in 3D were added to the biochip to check their long-term survival. Their functionality where characterized using the typical hepatic induced cytochrome-P450 (CYP1A) enzymatic activity profile in response to various toxic compounds in comparison to their already known analogue 2D activity. In order to increase the throughput we are developing a semi-automatic platform which will allow us to screen molecules on up to 12 biochips in parallel.

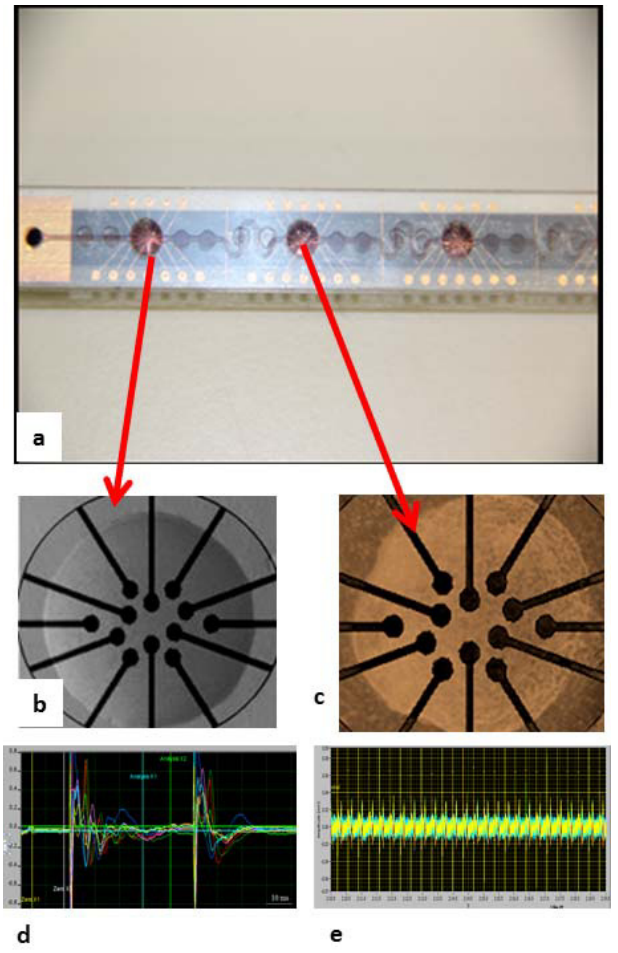

Fig. 4. (a) shows the under view of the biochip photography showing the 4 M.E.As in the four wells where Brain-like tissue (b) and cardiomyocytes cluster (c) where laid down onto MEAs. Electrophysiologcal recordings of the two tissues are respectively showed in (d) and (e).

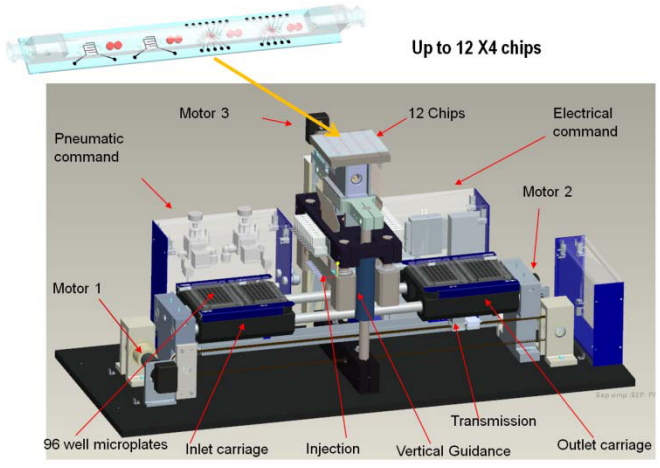

Fig. 5. Scheme of the multi-organs platform where up to 12 biochips can be tested in parallel for molecule screening.

4. Conclusion and perspectives

This human surrogate multi-organ biochip will enable the determination of toxicological profiles of new drug candidates. In addition, this chip should provide insight into inter-organ interactions resulting from exposure to pharmacological compounds, a capability which has not previously been demonstrated using previous *in vitro* systems. This system will thus be a more predictive tool in experimental pharmaceutical screening for efficacy and toxicity.

Acknowledgements

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