

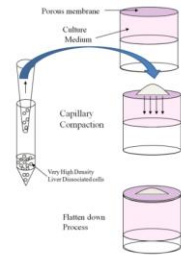
Introduction

Since human disease mechanisms and the general metabolism of humans are quite different from those of animals, using human stem cells will allow us to design better models of the human system to simulate more realistically *in vivo*-like responses to drug candidates as well as to determine the toxicity of newly developed or existing chemicals. Our project is thus in line with other approaches with the ultimate goal of determining more accurately safe exposure limits for work environments and consumer products.

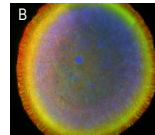
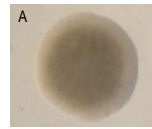
Materials and Methods

Tissue engineering

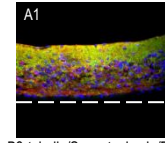
We are developing 3D culture systems for long term nervous tissue culture in an environment that more closely approximates that found *in vivo*. Embryonic stem cells from mouse and human origin were used to generate neural lineage cells. The protocol to generate 3D cultures consist by putting down a drop of culture medium that contains a high concentration of neural progenitor cells. The culture medium is attracted by capillarity through the membrane leaving a compacted cluster of cells onto the porous membrane. The resulting neural tissues can survive for prolonged periods, and maintain nervous tissue architecture.



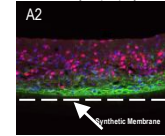
Method to generate 3D cultures from stem cell derived neural progenitors



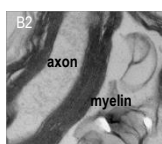
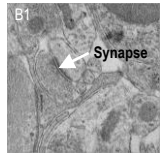
Macroscopically view of 3D neural cultures by light transmission (A) and by immunofluorescence stainings (B)



B3-tubulin/Synaptophysin/DAPI



NeuN/GFAP/DAPI



Histological Characterization of Neural progenitors derived from mESC (D3 line) 3D engineered tissues (immunocytofluorescence A1 and A2); (electron microscopy pictures B1 and B2)

Electrophysiological read-out:

Multi-Electrode Arrays (M.E.A.) system

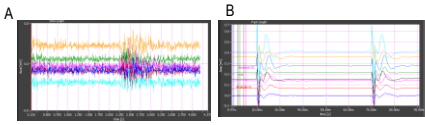
To determine if neurons were functional, neural tissues that had been cultured on pre-cut patches of membranes were transferred (after 2-3 weeks) to a multi-electrode array system and maintained at 33°C in aCSF. Tissues were positioned so that their different regions were in contact with electrodes. Spontaneous activity as well as evoked field potentials could be recorded from electrodes located in the different areas of the tissue.



BioCell 100 set-up (A) for electrophysiological monitoring and a Multi-Electrode Array (M.E.A.) cartridge (B). In C an engineered neural tissue is positioned onto a M.E.A.



Acquisition software for online and offline analyses



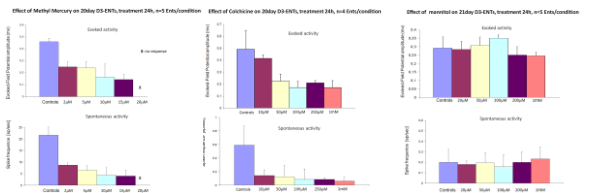
Spontaneous (A) and Evoked field potentials (B) recorded from mouse 21 day engineered neural tissue.

Results

A battery of pharmacological reference molecules were tested to assess the functionality of the neural tissue. We found that excitatory and inhibitory systems were present within the neural network. A first series of reference toxic compounds were tested on 3 week old engineered neural tissues. Preliminary results show that the reference toxic molecule from different chemical classes we have already tested triggered a decrease of spontaneous activity as well as evoked field potentials. In some cases epileptical activity could be observed prior to a dramatic decrease of the activity. We didn't record any modifications of spontaneous as well as evoked field potential activities when control reference molecules were added to the perfusion system.

	Spontaneous Activity	Evoked Activity
Kainic acid (2 µM)	+++	+++
Cocchine (10 µM)	+++	+++
Manganese chloride (100 µM)	+++	+
Methyl Mercury (2 µM)	+++	+++
Suprofen (µM to 500 µM)	-	-
Mannitol (1mM)	-	-

Table showing the effect of a first series of reference molecules tested on 3 week old mouse engineered neural tissues

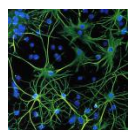


Dose-response Methyl-Mercury effect on 20 day mouse engineered tissue on evoked field potentials as well as spontaneous activities (A) Dose response Cocchine effect on 20 day mouse engineered tissue on evoked as well as spontaneous activities (B) No effect could be observed with Mannitol on 20 day mouse engineered tissue neither on evoked nor spontaneous activities (C)

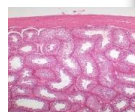
Perspectives

Detection and comparison of new markers in neural and testis 3D cultures

In addition to histological and electrophysiological approaches, we are planning to implement proteomic analyses to our platform to determine if new markers could be identify after exposure of toxic chemicals to 3D neural tissue cultures and we will compare the results to those obtained in 3D testis cultures. In a first step we will collect the supernatant and then perform a protein separation processing followed by a protein identification method using mass spectrometry. In a second step, we will also analyse intracellular content. Finally, we will determine if light spectroscopy approach will be helpful to follow up the metabolism of toxic compounds in neural and testis tissues.



Neural tissue



Testis tissue

