# Deposition chamber technology as building blocks for a standardized brain-on-chip framework

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## Abstract

Modeling of human brain connectomes is key to explore the structure-function relationship of the central nervous system (CNS). The comprehension of this intricate relationship will serve to better study the pathological mechanisms of neurodegeneration, and to perform improved drug screenings for neurological disorders. However, currently used in vitro modeling technologies are unable to represent the concurrent interconnectivity between myriad subtypes of neurons across multiple brain regions. Here, we present an innovative microfluidic design that allows the controlled and uniform deposition of various specialized neuronal populations within unique plating chambers of variable size and shape, used as organ-on-a-chip platforms for neuroscience research. By applying our design, we offer novel neuro-engineered Organ-on-Chip (OoC) microfluidics, also known as neurofluidic devices, which demonstrate the capacity to isolate, control, and modulate cellular environments, allowing to co-culture different cell types while these are fluidly isolated. The next step in the creation of Brain OoC is to couple a microfluidic architecture that can reconstruct complex interconnected neuronal networks, with a functional recording using multi electrode arrays (MEA). These advances provide essential enhancements to in vitro platforms in the quest accurately model the brain for the investigation of neurodegenerative diseases. Composed of multi-nodes interconnected with physiologically relevant structural connectivity patterns, we were able to create a specific models of CNS circuits as the direct way of the basal ganglia loop of the brain, involved in Parkinson disease. This loop is composed of five anatomic parts represented by five microfluidic compartments aligned on the device with respect to the converted required surface and linked using several arrays of microchannels. The activity of these networks can be record thanks to an electrophysiological recording system that has been specifically design to fit an even distribution throughout the compartments in 256 electrodes MEA.

## Introduction

Animal modelling is currently the gold standard when studying neuropatholgies, for lack of a better alternatives. In the last decade, **microfluidic based models** have started to appear, and have the potential to be able to model **human brain connectomes**, while offering scalable platforms potentially compatible with:

- high throughput automated technologies,
- high resolution microscopy,
- functional analysis.

Such models have to recapitulate **several key features**, such as the types of human cells used in relevant proportions, directional connectivity between these populations etc. One of these key factors, namely the relevant proportions between the various cell types, has remained unexplored.

Here, we present an innovative microfluidic design, called a **3D-Deposition Chamber**, that allows to control the **seeding of several neuronal populations** in microfluidic devices, in terms of: **number, density and, homogeniety**, while supporting their culture and maturation.

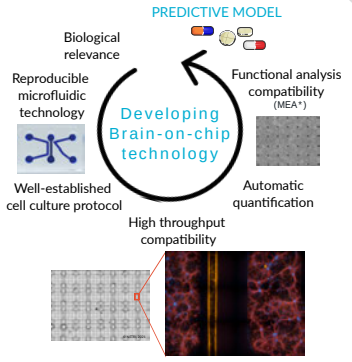


Fig. 1: Diagram of the development of Brain OoC for predictive modeling

## Results

### 1 What is a 3D-Deposition Chamber?

The device is constituted of 3D-Deposition Chambers (one or more) each connected to an inlet and outlet with precisely tailored hydraulic resistance to ensure control over the seeding of neurons. Each 3D-Deposition Chamber can then be connected via microchannels, ensuring control over the interconnectivity between the seeded populations and fluidic isolation.

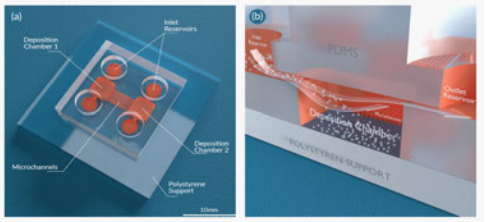


Fig. 2: (a) Artist view of the microfluidic design of brain OoC and (b) of a 3D-Deposition Chamber.

### 2 The 3D-Deposition Chambers allow reproducible control over the number of cells seeded and their homogeneity

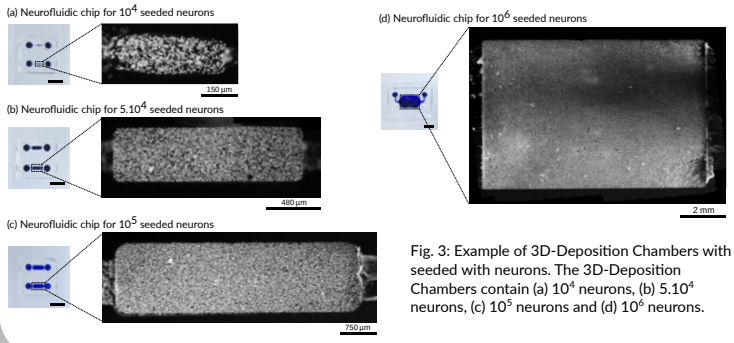


Fig. 3: Example of 3D-Deposition Chambers with seeded with neurons. The 3D-Deposition Chambers contain (a) $10^4$ neurons, (b) $5.10^4$ neurons, (c) $10^5$ neurons and (d) $10^6$ neurons.

### 3 3D-Deposition Chambers enable long term neuronal cultures with excellent viability

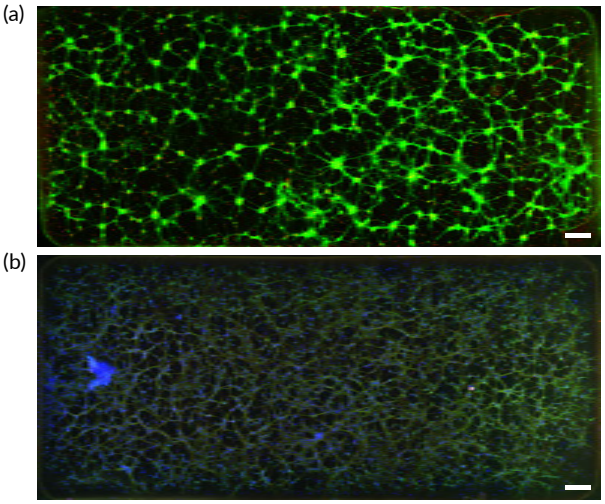


Fig. 4: Illustrative pictures of embryonic rat hippocampal cell culture at 18 DIV. (a) Staining with the LIVE/DEAD® Viability/ Cytotoxicity Kit for the assessment of alive cells (green) and dead cells (red). (b) Visualization of axons Tau (red) and dendrites MAP2 (green) against and and counterstained with DAPI (blue). Scale bars indicate 200 µm.

### 4 Example of 3D-Deposition Chambers to create minimalistic Basal Ganglia Loop models on MEA substrates

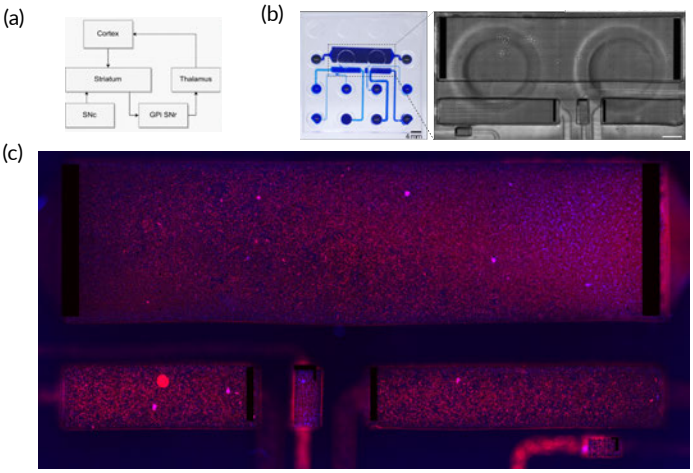


Fig. 5: 3D-Deposition Chambers to create a model of basal ganglia circuit on a chip. (a) Scheme representing the regions and connections within the in vivo circuit. GPi: Glubulus Pallidus internal, SNr: Substantia Nigra reticularis SNc: Substantia Nigra compacta. (b) Image of the reconstructed basal ganglia circuit on a chip (filled with blue ink). Inset: Transmission light microscope image of the MEA aligned on the neurofluidic architecture. (c) Immunofluorescent pictures of 18 DIV embryonic rat hippocampal with anti-MAP2 (Red) and with DAPI (blue). All images were obtained using a 10x objective.

## Conclusions and Perspectives

OoC technologies are state-of-the-art research tools that allow the construction of in vitro models with an accurate structural design at the organ level. This presented design, in combination with a multi-nodal patterning approach, brings major advances towards **modeling complex neural circuitry present in the intact brain** and provides the scientific community with standards matching industrial applications, allowing a **faster standardization and adoption of OoC** by the pharmaceutical industry. There are still challenges remaining for the validation of the OoC Basal Ganglia loop complete model, including accurate neural subtype seeding in each node, controlled directional connectivity between nodes and network wide electrophysiological recordings and connectivity mapping. This study describes an innovant microfluidic approach to create **improved neuro-engineered OoC devices via the seeding control of neurons into 3D-Deposition Chambers for the reconstruction of minimalistic brain connectomes**. We applied such innovative system to build an in vitro multi-nodal depiction of the basal ganglia circuit of the brain, whose dysfunction leads to neurodegeneration in Parkinson's disease.

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