Projects list

Block 1

Project 1: Generation and characterization of brain organoid of different regional identities and assembloids derived

Instructor: Sarah Frank (*FAU Erlangen-Nürnberg, Institute of Biochemistry, Germany*)

Experimental outline

The aim of the project is to generate iPSC-derived brain organoids and to learn the most important basic techniques to characterize them. We will first generate organoids using an unpatterned well-established protocol (Lancaster et al., 2013) that starts from iPSC, followed by an embryoid body phase, subsequent Matrigel embedding and fixation of organoids at different timepoints. We will use an additional protocol to pattern for specific regional identities, namely dorsal and ventral forebrain (Bagley et al., 2017). This will allow us to generate assembloids, fused organoids with different regional identity that mimic the interaction between different parts of the brain. Organoids of different ages will be fixed and cut in slices using a cryostat in order to perform immunohistochemical stainings to characterize the phenotype of the organoids. The output of the stainings will be assessed through imaging using fluorescent microscopes.

Project 2: Microfluidic methods for patterning brain organoids

Instructor: Richard O'Laughlin (*Perelman School of Medicine, University of Pennsylvania, USA*)

Background

Current methods for culturing brain organoids do not allow multiple regional identities to be stably, reproducibly and deterministically acquired within a single organoid. Microfluidic devices have the potential to deliver gradients of morphogens to brain organoids and thereby achieve predictable regional patterning.

Experimental outline

For this “organ on a chip” experimental section, the aim is to take students through the basics of designing, making and assembling microfluidic devices, show them how to culture brain organoids within them, and demonstrate the value of this approach compared to traditional culturing methods.

The techniques that will be covered include an overview of the photolithography process and micromachining for creating moulds, soft lithography with PDMS for making devices and bonding them to glass, setting up a microfluidic device (i.e. methods for loading cells and organoids, setting up syringes at inlet and outlet ports), methods for evaluating the performance of microfluidic devices and finally analysing results from microfluidics experiments.

Project 3: Different approaches to perform Electrophysiological recordings in mature human cerebral organoids

Instructor: Francesco Di Matteo (*Max Planck Institute for Psychiatry, Munich, Germany*)

Background

Human cerebral organoids recapitulate some unique features of human brain development and are increasingly being used as model systems to shed new light on the cellular mechanisms underlying the development of a variety of neurological diseases. However, the presence of functional neural network activities has only recently been demonstrated.

Experimental outline

This project aims to show different approaches to perform electrophysiological recordings and to explain how to analyze the recorded data.

In particular, the students will learn how to perform extracellular multielectrode array (MEA) recordings of mature cerebral organoids by using different approaches:

- Whole cerebral organoids (3D)
- Sliced cerebral organoids (3D)
- Dissociated cerebral organoids (2D-3D derived)

The students will also learn how to prepare the MEA plates and wells and plate the different samples, how to obtain organoids slices and how to dissociate them.

Project 4: Using single cell RNA sequencing to decipher cellular heterogeneity of cerebral organoids

Instructor: Fides Zenk (*BSSE, ETH Zürich, Basel, Switzerland*)

Experimental outline

This course will provide you with both theoretical as well as practical know-how on generating single cell RNA sequencing data from cerebral organoids using the 10x Chromium platform.

The theoretical part will cover how to design and perform single cell experiments and we will discuss latest technologies and possibilities to multiplex samples or perform screens with single cell transcriptomic read out.

In the experimental part of the course, we will dissociate cerebral organoids and prepare single cell and single nuclei suspensions. These suspensions will be the starting point for experiments involving FACS sorting or single cell genomics directly. You will also learn how to preserve the cells in order to store and process them later.

We will then use the 10x genomics workflow to generate single cell RNA libraries for next generation sequencing and perform all necessary quality controls to submit the samples for sequencing.

The last part will include a dry-lab session where we will use your own or published data to perform first quality controls and analysis steps to understand single cell transcriptomics data.

Project 5: Transplantation of hPSC-derived brain organoids into mouse brain

Instructor: Abed Mansour (*The Hebrew University of Jerusalem, Israel*)

Background

Recent advances in human pluripotent stem cells (PSCs) technology and subsequent differentiation into multiple 3D organotypic neural structures, termed brain organoids, have provided a unique opportunity to investigate the physiology and the pathophysiology of the human brain in a new way. However, in vitro culture models lack the cellular interactions, neurovascular system, and the in vivo physiological environment of the living brain. We have recently established a method to generate an organoid-based chimeric model that allows vascularization and functional integration of human brain organoids in vivo.

Experimental outline

The objective of this mini-project is to learn how to establish this chimeric system through transplantation of human PSCs-derived brain organoids into rodent brain. The students will be trained in surgical procedure of grafting brain organoids into mouse brain. They will assess the success of transplantation using fluorescent microscopy. Students will then learn how to dissect the grafted brain and process the tissue using cryosectioning. Finally, if time allows it, immunofluorescence staining and confocal imaging will be used to analyzed the grafted organoids using multiple markers.

Techniques

- Surgical procedure of organoid transplantation
- Animal perfusion
- Mouse brain dissection and freezing
- Brain cryosectionin using cryostat
- Immunofluorescence
- Imaging using confocal microscopy
- Image analysis

Project 6: A sneak peek into retinal organoids

Instructor: Giovanna Brancati (*BSSE, ETH Zürich, Basel, Switzerland*)

Background

Retinal organoids beautifully resemble the human neural retina, with its neurons (photoreceptors, horizontal, bipolar, amacrine and ganglion cells) and glial cells (Müller glia). The cells in the organoids arrange into a stereotypical laminar structure thereby recapitulating the nuclear and synaptic layers observed in the human retina.

Experimental outline

During this practical course, we will generate retina organoids from human induced pluripotent stem cells (iPSC) and go through some of the key steps of the protocol.

We will also use immune-histochemistry and confocal microscopy to visualize the major cell types at different developmental time points.

By the end of the course, the students will be familiar with the retina organoid protocol and will be able to grow their own organoids, recognize their developmental age, the retina cell types and structures.

Block 2

Project 1: Generation and characterization of brain organoid of different regional identities and assembloids derived

Instructor: Federica Furlanetto (*FAU Erlangen-Nürnberg, Institute of Biochemistry, Germany*)

Experimental outline

The aim of the project is to generate iPSC-derived brain organoids and to learn the most important basic techniques to characterize them. We will first generate organoids using an unpatterned well-established protocol (Lancaster et al., 2013) that starts from iPSC, followed by an embryoid body phase, subsequent Matrigel embedding and fixation of organoids at different timepoints. We will use an additional protocol to pattern for specific regional identities, namely dorsal and ventral forebrain (Bagley et al., 2017). This will allow us to generate assembloids, fused organoids with different regional identity that mimic the interaction between different parts of the brain. Organoids of different ages will be fixed and cut in slices using a cryostat in order to perform immunohistochemical stainings to characterize the phenotype of the organoids. The output of the stainings will be assessed through imaging using fluorescent microscopes.

Project 7: Generation and characterization of specialized organoids: cerebral and choroid plexus organoids

Instructor: Laura Pellegrini (*MRC-LMB, Cambridge, UK*)

Background

Our brain is immersed in the cerebrospinal fluid (CSF), an important source of nutrients and signalling molecules that guide brain expansion. The choroid plexus (ChP) is the tissue responsible for cerebrospinal fluid (CSF) production, and functions as a barrier regulating entry of compounds and nutrients into the brain. The ChP-CSF system plays key roles in brain development, homoeostasis and in clearance of toxic by-products. Abnormalities in CSF can result in neurological diseases such as hydrocephalus, autism and schizophrenia.

Human ChP development and CSF composition are poorly understood due to a lack of experimental access to the human ChP and to the absence of authentic human CSF from in vitro models.

To study the development and function of the human ChP, we can now generate an organoid model derived from pluripotent stem cells to develop “human ChP in a dish” with accurate architecture and function. ChP organoids reliably display key morphological and functional features of human ChP and develop fluid-filled compartments isolated from the surrounding culture media. ChP organoids form a tight barrier that exhibit the same selectivity to small molecules as in vivo representing an easily tractable system to study the key functions of this brain tissue.

Experimental outline

During this project, students will learn the key steps to generate cerebral and choroid plexus organoids. We will perform morphological comparisons between the two types of organoids using techniques such tissue embedding and sectioning followed by analysis by immunofluorescence to visualize the different cell types present in the organoids as well as specific markers differentially expressed in the tissue. Finally, we will perform choroid plexus

organoid dissociation and reaggregation experiments to test whether the choroid plexus barrier is able to reform.

Techniques

- Early steps of generation of cortical and choroid plexus organoids
- Late steps of cortical and choroid plexus organoid protocol
- Tissue analysis
- CSF sampling from choroid plexus organoids, choroid plexus organoid
- Data analysis

Project 8: Revealing neuronal activity in brain organoids using microelectrode array (MEA)

Instructor: Yan Hong (*Perelman School of Medicine, University of Pennsylvania, USA*)

Experimental outline

MEA is a neuronal spike recording system that has been widely used to measure neuronal activity. The aim of this project is to show students that MEA is a useful tool for analyzing functions of neurons. First, I will show students how to generate organoid slices. Second, I will teach them how to prepare samples before recording, including coating MEA plate, plating sliced organoids, and culturing them on MEA plate for one week. Finally, I will show them how to record and analyze the electrophysiologic properties, such as spikes and network bursting events. The main techniques that I will use are slicing organoids using vibratome, MEA recording, and data analysis.

Project 9: Single cell analysis in brain organoids

Instructor: Maren Büttner (*DZNE, Bonn, Germany*)

Experimental outline

In this computational project, students will learn current best practices and approaches for analyzing single-cell RNA-seq data using the analysis framework scanpy and a selection of R-based packages. The instructor will introduce the computational basis for the respective methods, combined with practical hands-on sessions to familiarize with the computational procedure. That also includes the installation and setup of the different analysis environments. The students will directly work on single-cell RNA-seq data obtained from brain organoids. In particular, the course covers quality control, normalization and scaling, feature selection and integration of multiple samples, thereby monitoring technical effects. Then, the students will identify cell types and their characteristics through clustering and examining differences in gene expression patterns. Further, the course will cover downstream analysis questions such as dynamical processes captured via trajectory inference and RNA velocity analysis, differential expression testing, gene set enrichment, and evaluation of compositional shifts. Many of these approaches are directly applicable on the experimentally available data and the students will address different downstream analysis parts individually. Ultimately, the course intends to provide a blueprint for future single-cell RNA-seq data analysis, raise awareness for statistical pitfalls, and aims to build up competence and confidence for the analysis.

Project 10: Investigate the Neurogenesis of Transplanted Forebrain Organoids In Vivo with Immunostaining

Instructor: Ting Zhao (*Perelman School of Medicine, University of Pennsylvania, USA*)

Background

Brain organoids model congenital brain conditions and neuropsychiatric disorders, and have promoted the study of brain development. Although recapitulate certain aspects of brain architecture, brain organoids cultured in dish are immature. Transcriptomic studies revealed that these organoids mimic the late second trimester of human fetal development approximately. To facilitate the maturity of organoids, the brain organoids are transplanted into the rodent brains.

Experimental outline

In this project, I will teach students how to perform immunostaining to verify the quality of transplanted brain organoids, and detect neurogenesis of transplanted organoid in rodent brains. Immunostaining technique will show students human neural stem cells, deep-layer and upper-layer cortical neurons, as well as rodent immune response to the transplanted organoid. The techniques used in this project including 1) Perfuse animal brains transplanted with organoids, 2) Slice the brains with cryostat or vibratome, 3) Immunostaining and 4) Imaging with confocal microscope.

Project 11: To Fate or Not To Fate: live-cell imaging of neural progenitor cells to study cell fate decisions in the human developing neocortex

Instructor: Clarisse Brunet (*Institut Curie, Paris, France*)

Background

The human cerebral cortex is composed of diverse cell types and has expanded dramatically through evolution. While increased and diversified progenitors play a key role in cortical development and expansion, the mechanisms by which such cellular heterogeneity arises remain largely unknown. Here, using live-cell imaging in combination with techniques that allow specific labeling of distinct cell types, we aim to come closer to elucidating this phenomenon, which can help to shed light on certain neurodevelopmental disorders and cortical evolution.

Experimental outline

During this project the participants will learn the basis of live-cell imaging, from sample preparation to handling a spinning-disk confocal microscope. In brief, we will slice thick sections of human brain organoids (8-10 weeks old) and infect them with retroviruses to deliver GFP to dividing cells. The slices will be then culture at the air-liquid interface for 2 days to enhance proliferation and live imaged for another 2 days. At the end of the acquisition, the slices will be fixed, immunostained and imaged. The resulting acquisitions will be used to characterize neural progenitor cells and the generation of the different cell types populating the human developing neocortex.

Techniques

- Tissue slicing (vibratome) and culture at the air-liquid interface
- Viral infection (transduction)
- Live-cell imaging (spinning-disk confocal microscope)
- Basic image processing using Fiji ImageJ.