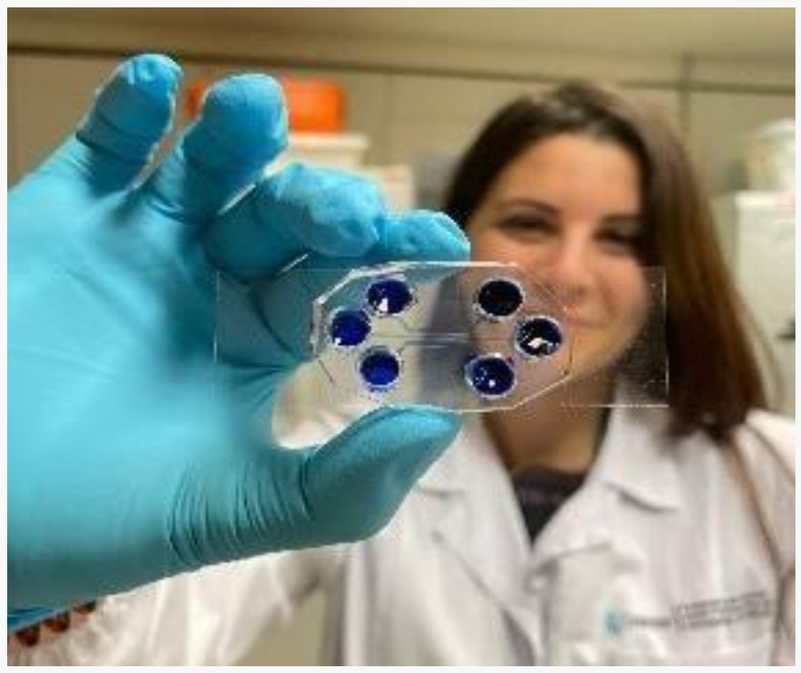
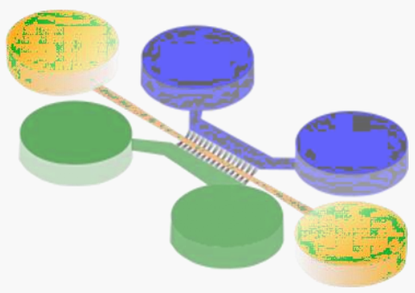
BRAIN-ON-A-CHIP TECHNOLOGY FOR BIOMEDICAL RESEARCH



Abstract

Human-based Brain-on-a-Chip technology is a viable solution for studying the human biology of neuronal connectivity in normal and disease conditions. avantdrug's Brain-on-a-Chip is a new platform based on the combination of different human pluripotent stem-cell-derived neurons. The flexibility of our platform allows for the combination of different neural cell types to assess the maturation and functionality of neuronal connections.

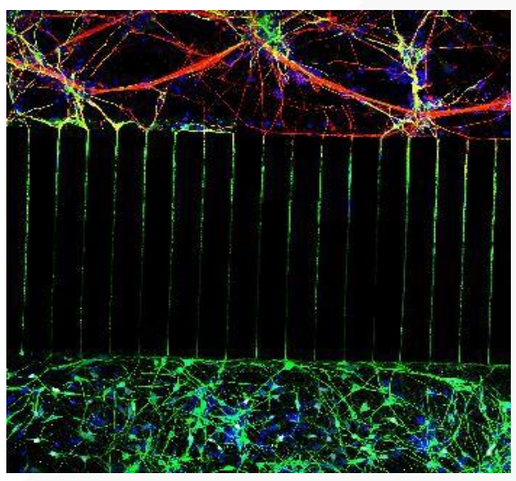
- ✓ avantdrug's multi-chamber brain-on-a-chip design allows the combination of up to three parallel neural differentiations.
- ✓ Different seeding strategies combining several developmental stages allow for the analysis of afferent connections on neuronal maturation.
- ✓ The directional axonal growth of avantdrug's Brain-on-a-Chip mimics the brain's neuronal networks.
- ✓ The platform permits studies of functional neurological processes in health and disease to test drug effects and toxicity.



Applications of the avantdrug Brain-on-a-Chip

1. Neurodevelopmental studies and synaptic plasticity.
2. Modelling of human neurodegenerative disorders to understand biological mechanisms.
3. Drug testing to assess impact on development and maturation of neuronal connections.
4. Toxicological studies during neurodevelopment and of neuronal connectivity.

High-content analysis

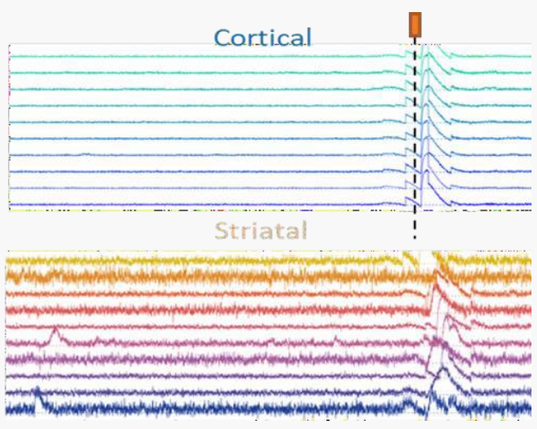


- ✓ **Compatible with high-content analysis:** simultaneous characterization and quantification of different cell types in each chamber.
- ✓ **Establishment of and impacts on connections can be measured:** combination of functional and morphological methods to characterize connectivity.
- ✓ **Detection of synaptic connections by monosynaptic anterograde tracer:** analysis of synaptic connections by combination of viral-based monosynaptic tracer.

Drug testing & toxicology

- ✓ **Drug testing:** testing the efficacy of new drugs on neuronal connectivity and synaptic function, applying new drugs to control or disease-derived human cells to analyze their therapeutic effects.
- ✓ **Developmental toxicology:** analysis of the toxicity of new drugs on neuronal function, connectivity, and synaptic function.

Analysis of synaptic function



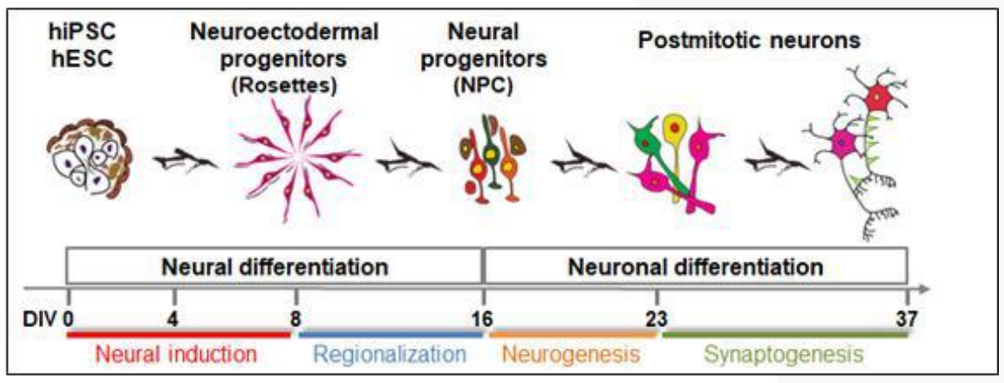
- ✓ **High-throughput functional analysis:** compatible with high-throughput calcium imaging to assess neuronal functionality and network formation.
- ✓ **Drug testing:** capable of performing functional pharmacological studies of neuronal connectivity in independent chambers.
- ✓ **Evoked neuronal response:** allows analysis of drug-evoked responses or optical stimulation using optogenetics of individual chambers, to test the putative positive and negative effects of drugs on evoked responses.
- ✓ **RNAseq:** compatible with genetic characterization using bulk-RNAseq or single cell/nuclei- RNAseq as well as the genetic modification of neural cell types at different stages.

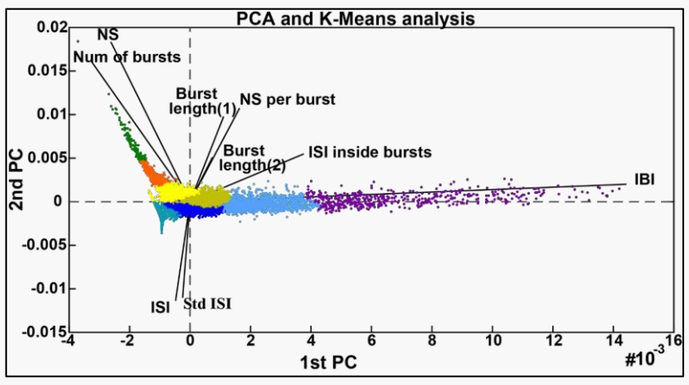
BIBLIOGRAPHY

- Comella-Bolla et al., Human Pluripotent Stem Cell-Derived Neurons Are Functionally Mature *In Vitro* and Integrate into the Mouse Striatum Following Transplantation. *Mol Neurobiol*. 2020 57:2766-2798.
- Molina-Ruiz et al., Standardization of Cell Culture Conditions and Routine Genomic Screening under a Quality Management System Leads to Reduced Genomic Instability in hPSCs. *Cells.* 2022 11:1984.
- Introna et al., Reconstructing cortico-striatal network in a chip deciphers the role of cortical inputs on human striatal development. *In prep.* 2023.

We ensure all projects are compliant with applicable UNE-EN-ISO 9001:2015 and/or GLP guidelines, and the Creatio Quality System.

NEUROTOXICOLOGICAL AND NEURODEVELOPMENTAL STUDIES USING HUMAN MODELS



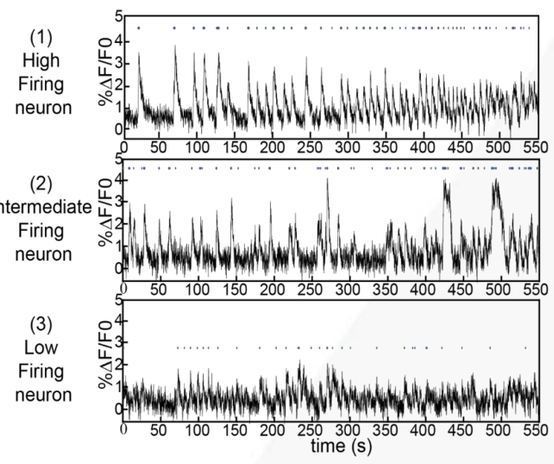


Standardized cell culture

Built on more than 20 years of experience in the expansion and differentiation of human pluripotent stem cells (hPSCs), our protocol for neuronal differentiation has been reproduced more than 500 times with the strictest quality controls:

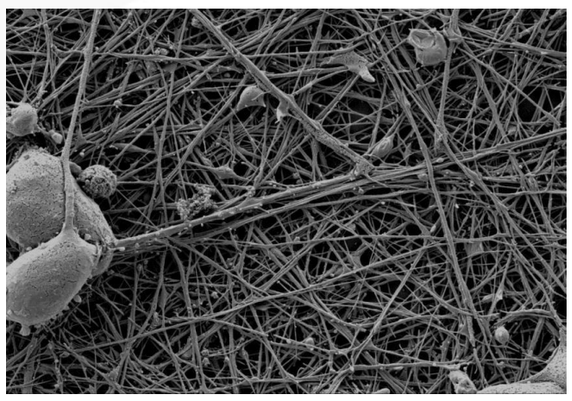
- ✓ **Karyotype stability:** we establish regular controls on genome abnormalities, testing chromosomic alterations by Q-band analysis every 10 passages and before starting each differentiation process, and by CGH array 20–30 passages.
- ✓ **Reproducibility:** we have tested our protocol in more than 10 different hPSC lines, including embryonic and induced hPSCs, obtaining a protocol reproducibility above 95% consistency.
- ✓ **Traceability:** we operate under ISO 9001:2015 and the Creatio Quality System, guaranteeing the traceability of all stem cell passages, the products used, and the results obtained.

High-throughput analysis



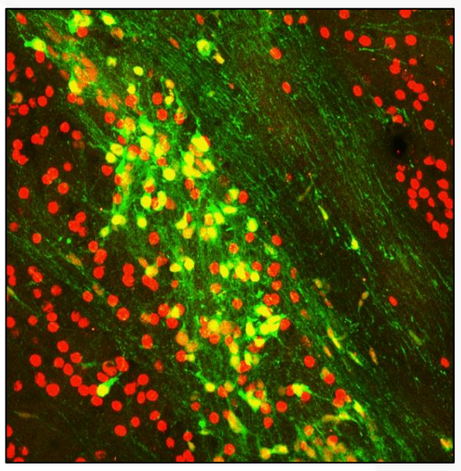
- ✓ **Gene expression profiles:** our OpenArray QPCR-based platform analyzes the expression of (I) 110 key genes at different developmental stages and (II) 160 genes associated with synaptic functionality (synaptic receptors, neuronal channels, and second messengers).
- ✓ **High-content analysis:** a wide range of specific markers for each developmental stage, from pluripotency to mature neurons and neurotransmitters.
- ✓ **Calcium imaging:** spontaneous and evoked neuronal response to chemicals or optical stimulation that allows the classification of neurons based on activity.

Connectivity analysis



- ✓ **Electron microscopy:** analysis of connectivity formation by scanning electron microscopy at different development stages.
- ✓ **SNARE analysis:** we perform SNARE characterization by semi-quantitative western blot.
- ✓ **Immunocytochemistry of synaptic proteins:** fine colocalization of synaptic proteins permits the analysis of disturbances of regular function.
- ✓ **Extracellular vesicle characterization:** we investigate the morphological alteration of subcellular organelles.

Chimeric human-mouse model



- ✓ **Cell transplantation:** transplantation of hNPCs from control and Huntington's Disease patients into the striatum of new-born mice.
- ✓ **Cell integration, differentiation and axonal projection:** transplanted hNPCs differentiate into striatal neurons and send axonal projections towards and establish synaptic connexions within the host basal ganglia circuitry.

Drug tesing and toxicology

- ✓ **Drug testing:** efficacy testing of new drugs along the neurodevelopmental process. New drugs can be tested at all developmental stages in control or disease-derived human cells.
- ✓ **Developmental toxicology:** drug toxicity analysis in different neuronal developmental stages. High-throughput analysis of neuronal development and its response to new drugs.

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- HD iPSC Consortium. Developmental alterations in Huntington's disease neural cells and pharmacological rescue in cells and mice. *Nat Neurosci*. 2017 20:648-660
- Telezhkin *et al*., Kv7 channels are upregulated during striatal neuron development and promote maturation of human iPSC-derived neurons. *Pflugers Arch.* 2018 470:1359-1376.
- Comella-Bolla *et al*., Human Pluripotent Stem Cell-Derived Neurons Are Functionally Mature In Vitro and Integrate into the Mouse Striatum Following Transplantation. *Mol Neurobiol*. 2020 57:2766-2798.
- Molina-Ruiz *et al*., Standardization of Cell Culture Conditions and Routine Genomic Screening under a Quality Management System Leads to Reduced Genomic Instability in hPSCs. *Cells.* 2022 11:1984.
- Miguez, A., Gomis, C., Vila, C. et al. *Soluble mutant huntingtin drives early human pathogenesis in Huntington's disease*. *Cell. Mol. Life Sci* 2023. 80, 238.

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PRECLINICAL REGULATORY SAFETY STUDIES

avantdrug offers preclinical studies of your product under development for regulatory approval:

avantdrug is the preclinical studies area of Creatio, dedicated to the validation of new technologies and healthcare products under development for their regulatory approval.

With extensive experience in the field, our skilled professionals provide private and public sector clients with a wide range of preclinical studies conducted under high quality standards, ISO 9001:2015 and/or GLP quality standards, as well as technical and scientific advisory services.

avantdrug aims to become the foremost preclinical research center of its kind, setting a benchmark for preclinical studies that promote the timely and cost-effective launch of innovative, safe and effective products for the benefit of the society.

TOXICITY STUDIES

✓ Toxicity *in vitro*:

- *In vitro* skin irritation & corrosion tests (OECD 431 & 435, 429, ECVAM DB-ALM 47)
- Balb/c 3T3 cell phototoxicity assay (OECD 432)
- *In vitro* cytotoxitity tests (HepG2, A549, 3T3, Caco-2) (INVITTOX 17 & 64)
- Cytotoxiticy in primary hepatocytes of rats & other species (INVITTOX 20)
- Cell transformation assay (CTA) (ENV/JM/MONO(2015)18)
- Screening assay for different cell types
- Phototoxicity assay (OECD 432)

✓ Reproduction & developmental toxicity:

- Fertility
- Reproduction/developmental toxicity screening (OECD 421)
- Embryonic stem cell test *in vitro* (ECVAM DB-ALM 113)
- Repeat-dose toxicity study combined with reproduction/ developmental toxicity screening test (OECD 422)
- Prenatal development (OECD 414)
- 1- and 2-generation reproduction toxicity study (OECD 415 & 416)

CLINICAL PATHOLOGY

- Histopathology service for necropsy, histological processes, and evaluation
- Hematological & biochemical determinations
- Histochemical & immunohistochemical determination in tissues
- Coagulation profiles

✓ Genotoxicity:

- Bacterial reverse mutation assay (Ames) (OECD 471)
- *In vitro* mammalian cell gene mutation test (OECD 476)
- *In vitro* and *in vivo* micronuclei assay (OECD 487 & 474)
- *In vitro* and *in vivo* Comet assay (OECD 489)

✓ Local tolerance:

- Acute local tolerance & repeat-dose study
- Local lymph sensitivity assay (OECD TG 442A)
- *In vitro* skin sensitization & absorption test (OECD TG 442E, OECD 406 & 428)

✓ Toxicity *in vivo* & carcinogenicity:

- Acute toxicity studies (OECD 402, 404, 405, 420, 423 & 425)
- Repeat-dose toxicity (OECD 407, 408, 409, 410, 411 & 452)
- Carcinogenicity assay (OECD 451) combined with chronic toxicity (OECD 453)
- Behavior evaluation

ADME, PK & PK/PD STUDIES

- Absorption, distribution, metabolism & excretion (ADME) studies
- Pharmacokinetic studies: lineality, bioavailabity & bioequivalence
- Pharmacokinetic/pharmacodymanic relationship studies
- Kinetic studies of new forms of dosage and local tolerance
- Toxicokinetic evaluation

INTEGRATED SERVICES

- Elaboration of preclinical roadmap
- Regulatory requirements assessment
- Expert reports
- Optimal procedure design to reduce cost and timings
- Expert reports
- Scientific & technical advice
- Support with documentation for regulatory authorities

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