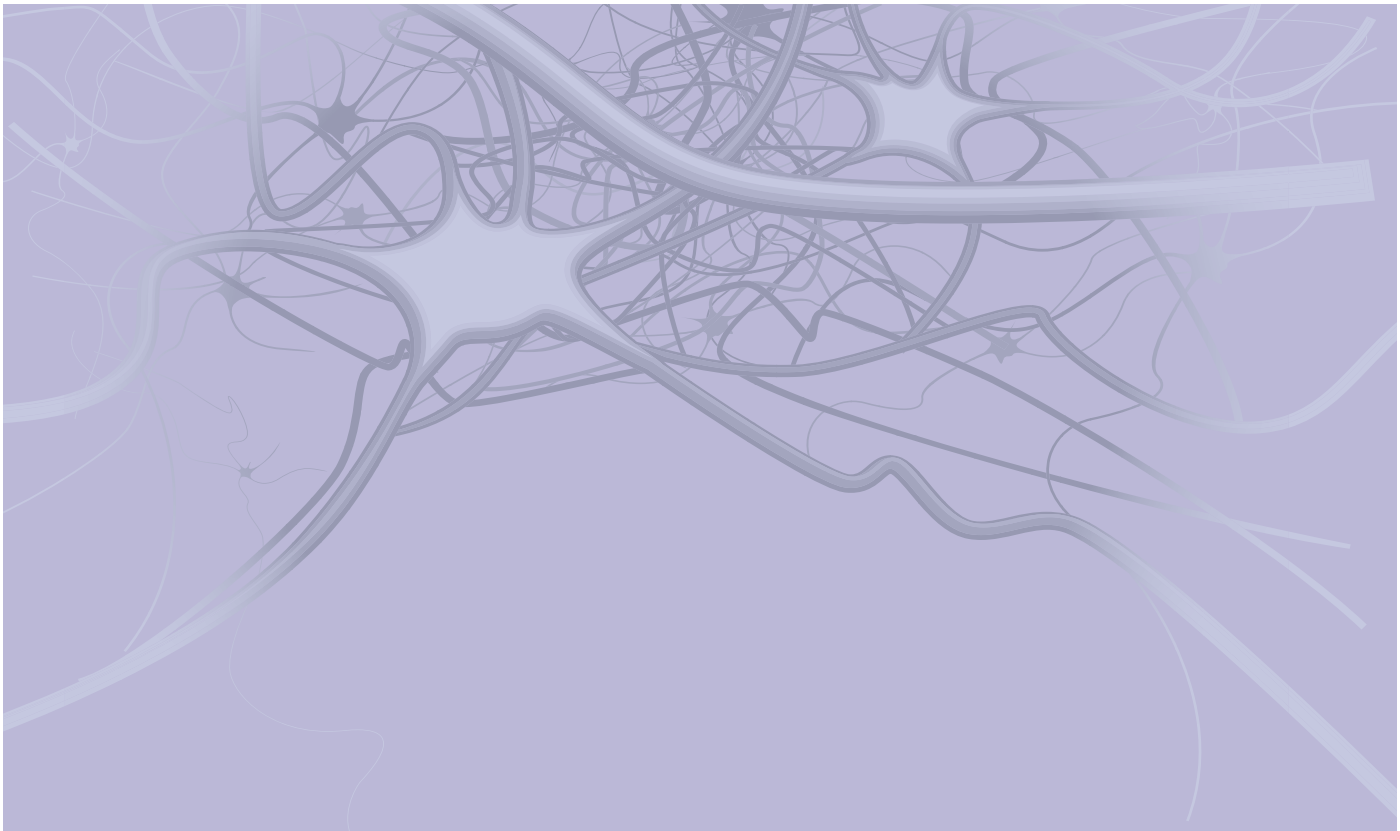
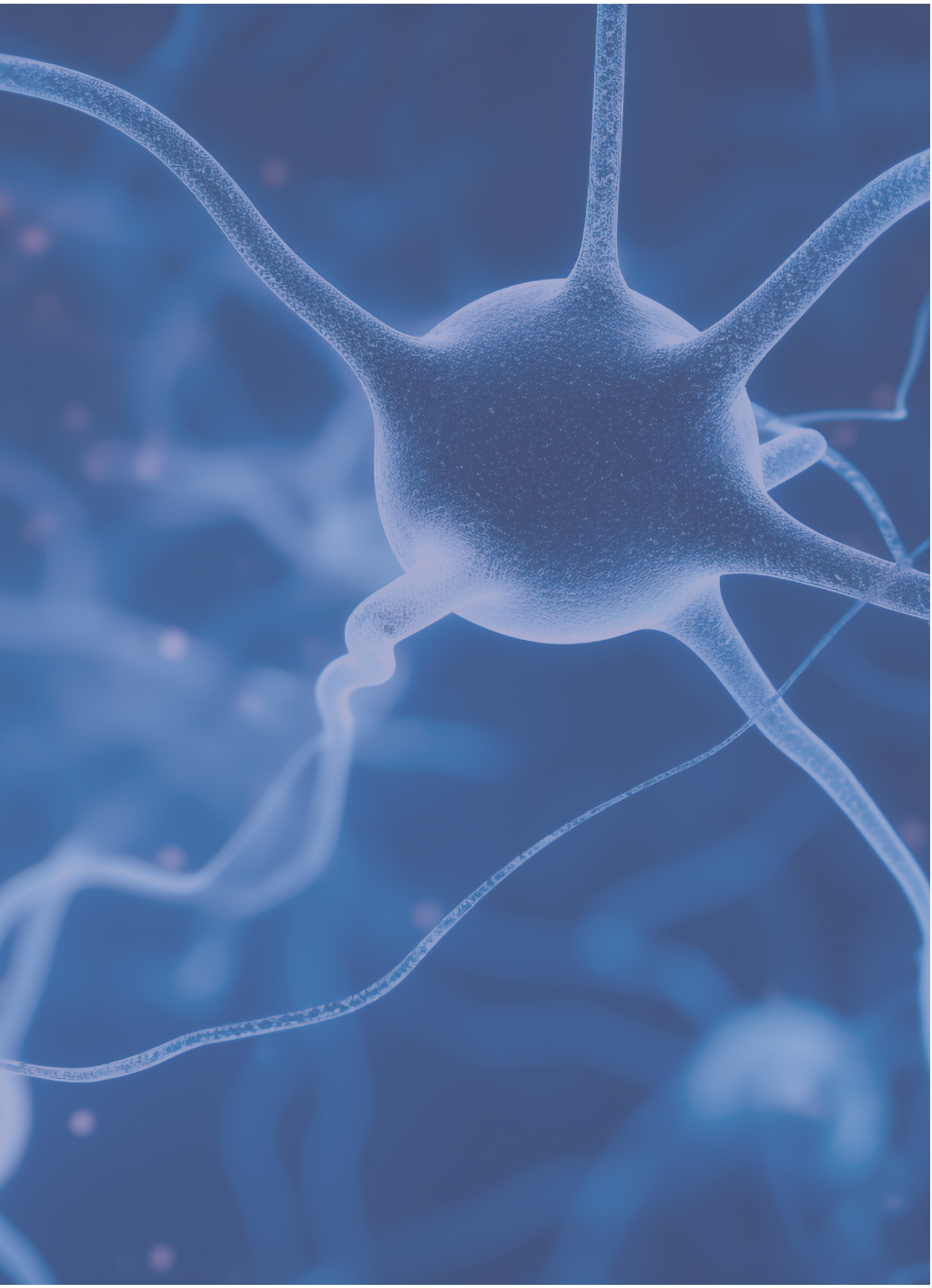


WHITE PAPER

Breaking the Bias:

Addressing the challenges facing optimal neural organoid development

CELVIVO
STRESS-FREE 3D™



01.
Introduction

One of the major goals of neurobiology research is to achieve detailed understanding of nervous system development, and to clarify the mechanisms underlying various nervous system disorders. Unfortunately, research is often augmented by limited access to healthy and diseased human brain tissue for studies.

To circumvent this issue, researchers have turned to animal models and in vitro cell culture systems, with varying success.

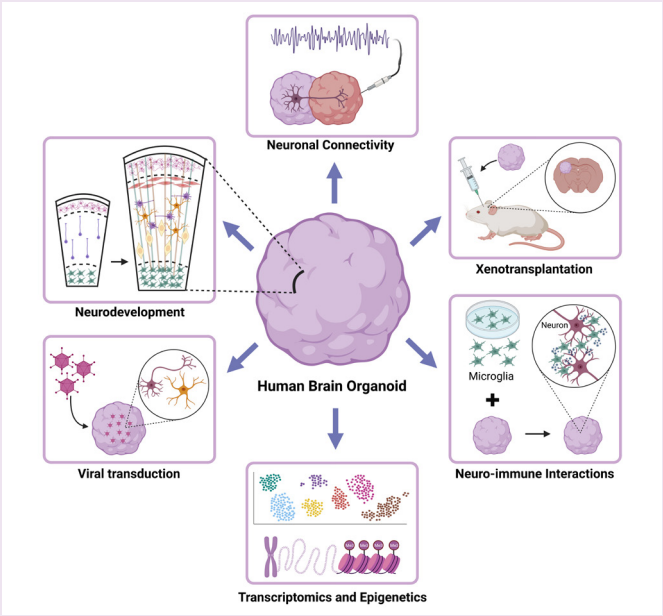


Figure 1. Human brain organoids are accessible cellular models. *Brain organoids can be used for different down-stream applications including neuronal connectivity, xenotransplantation, neuro-immune interactions, transcriptomics and epigenetics, viral transduction, and neuronal development. Illustration is inspired by "Building Models of Brain Disorders with Three-Dimensional Organoids" 2018, Neuron Review by Neal D. Amin and Sergiu P. Pasca (https://doi.org/10.1016/j.neuron.2018.10.007).*

Animal models fail to accurately recapitulate the species-specific aspects of the human brain, due to substantial genomic differences, while traditional, adherent / 2D cell culture models are incapable of mimicking the complex tissue architecture and cellular transport mechanisms that exist in 3D microenvironments. Thus, there exists a significant gap in the field: the need for a model that can accurately replicate in vivo conditions to study human brain development and maturation in a healthy as well as diseased environment.

A remarkable advancement towards mitigating this gap has been the development of three-dimensional (3D) neural cultures. Induced pluripotent stem cells reprogrammed from human somatic cells can be differentiated into specific neural lineages, giving us the opportunity to model human neural development in a relevant in vitro system. This process requires the development of 3D aggregates of stem cells, grown with the assistance of extracellular matrix or in a suspension format, which are then guided to recapitulate neural features of interest, with the help of various growth factors and nutrients. 3D brain cultures are thus better suited to provide physiologically relevant models that can mimic tissue architecture, cellular diversity as well as cell-cell interactions in a human derived system.

Further technical advancement has revealed exposure to instructive signals can guide neural progenitor aggregates to self-organize and self-pattern to become both regionally specific and functionally relevant. These are referred to as regionalized neural organoids, capable of demonstrating the cellular, molecular, and anatomical characteristics of the neural domain they are designed to represent. Protocols to develop neural organoids of forebrain, midbrain, hindbrain, retinal and cerebral specifications have been well-established, indicating significant progress that has been made in this work.

02.
3D cell culture technologies and challenges

As previously stated, there are two primary methods for generating neural organoids, either with the assistance of an exogenous matrix, or independent of matrix in a suspension format. Artificial matrices are gelatinous protein mixtures obtained from various sources, including tumour cells, designed to act as a basement membrane for cells to adhere to. Given their varied and non-homogenous sources, these matrices are rarely well defined, and are known to introduce much variability in experimental results. Additionally, matrices are designed to operate in a static environment, where cell aggregates will adhere to the matrix as an artificial basement membrane. Due to its static nature and resulting lack of media flow, gas exchange and nutrient consumption will be inefficient, causing cells across the aggregate to starve and suffer from suffocation. This may result in an aggravated necrotic or hypoxic core build-up, in addition to structurally non-uniform cellular aggregates.

The alternative to introducing artificially derived matrices to 3D neural aggregates is to employ matrix-free, dynamic, suspension-based methods. These include spinner flask systems, bioreactors, and orbital shakers. Currently, the systems commonly employed for dynamic cell culture generate high shear stress on cells, due to the requirement of high speeds of rotation. Higher speeds result in an agitative atmosphere for the cells, creating high shear stress which may cause increased cell death, generation of excessive debris due to greater death and the production of uneven, non-uniform cultures. Additionally, most bioreactor systems rely on an agitation component which is placed within the cell culture chamber of the bioreactor, such as a rotating wheel. These components act as a physical barrier within the chamber, adding more stress on the culture media and cells, further propagating shear stress and premature cell death.

Figure 2. ClinoStar and ClinoReactor by CelVivo Aps, Denmark. *The ClinoStar is a clinostat standalone CO_2 incubator which allows you to culture cells in 3D on six individual axes. The ClinoReactor is an all-in-one bioreactor with a humidification system for minimizing the risks of infections and keeping the volume constant in the incubation chamber. The vents in top of the ClinoReactor ensures a constant flow of air over the membrane allowing CO_2 to be in equilibrium with the media inside the cell chamber.*
For more information, please visit www.celvivo.com.

03.
ClinoStar and human 3D brain organoids

The ClinoStar is designed to specifically address these above-mentioned challenges. The ClinoStar is a rotary bioreactor system which uses constant rotation of bioreactors at gentle speeds to create a simulated microgravity environment.

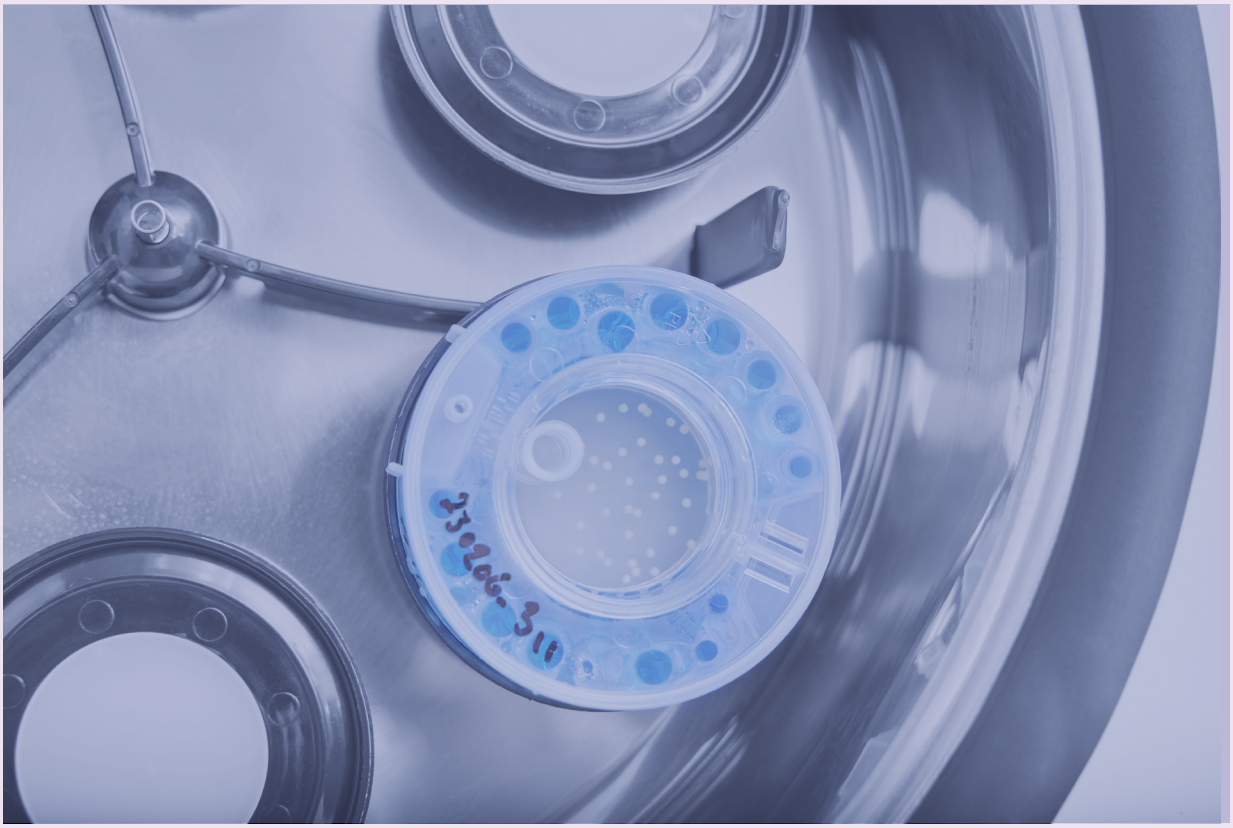
This dynamic system keeps cells in suspension and allows them to form aggregates without the use of excessive force or artificial matrices.

Due to the lack of a directional gravitational pull experienced by the samples in the ClinoStar, the cells enjoy a longer, more stable growth phase which more closely mimics in vivo standards.

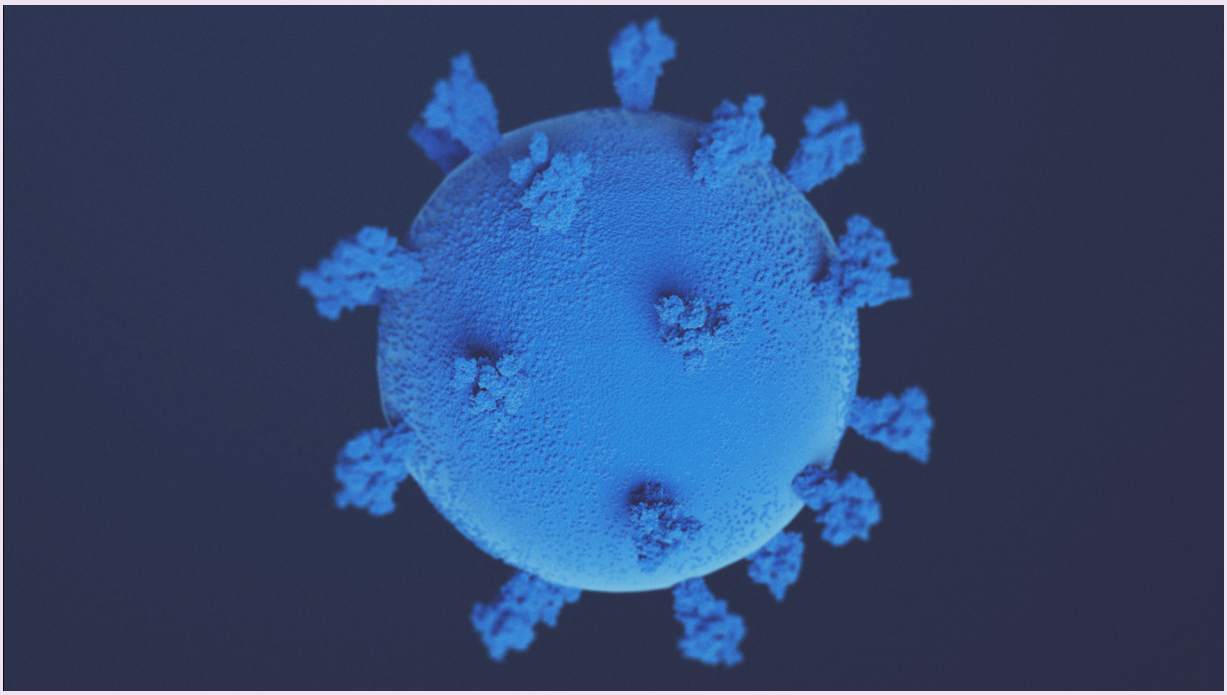
The dynamic nature of the system allows constant media flow, which prevents formation of a nutrient depletion zone around the periphery of each individual aggregate.

Efficient gas exchange and nutrient delivery prevents formation of premature necrotic cores and creates an environment that can support long term growth, which is pivotal for brain organoids.

Human CNS development is a lengthy and temporally defined process, and a culture method that can promote longevity are critical to mimic in vivo brain development conditions.



04.
How to use induced pluripotent stem cells to generate cortical brain organoids



Our selected publication "Omicron BA.5 infects human brain organoids and is neuroinvasive and lethal in K18-hACE2 mice", 2022 describes how to use human induced pluripotent stem cells to generate cortical brain organoids.

Are SARS-CoV-2 viruses evolving to become less pathogenic over time?
Not according to a recent publication from the lab of Andreas Suhrbier. In this study, scientists used the CelVivo ClinoStar to develop human induced pluripotent stem cell (hiPSC) derived cortical organoids and probe the pathogenicity of three different variants of SARS-CoV-2. The scientists found that the newer Omicron variant, BA.5, infected human cortical brain organoids significantly more than both the older Omicron variant, BA.1, and the original ancestral isolate.

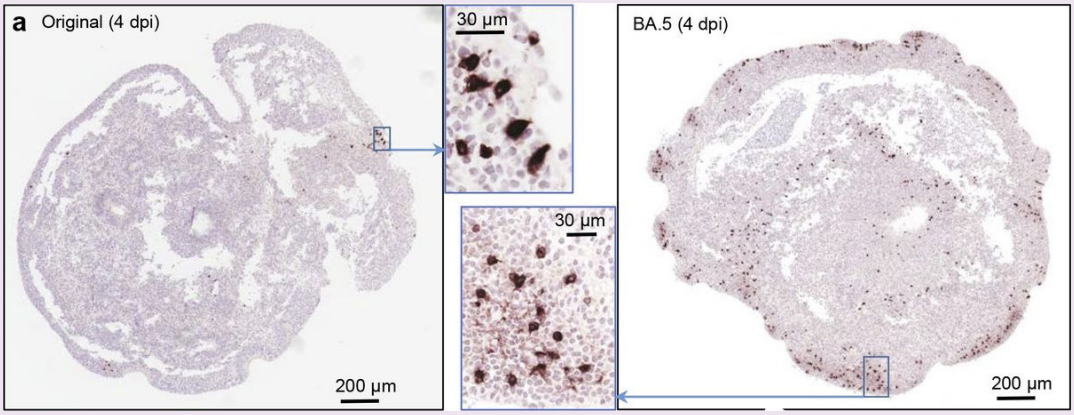


Figure 3. Cortical brain organoids derived from human induced pluripotent stem cells. *P. 8: Immunohistochemistry of sectioned cortical brain organoids previously infected with 4 dpi original ancestral or BA.5 using anti-spike monoclonal antibody. It is clear from the degree of staining that the BA.5 is the most pathogenic. P. 9: photograph of cortical human brain organoids grown using the CelVivo ClinoStar system. Approximate size: 2-3 mm.*

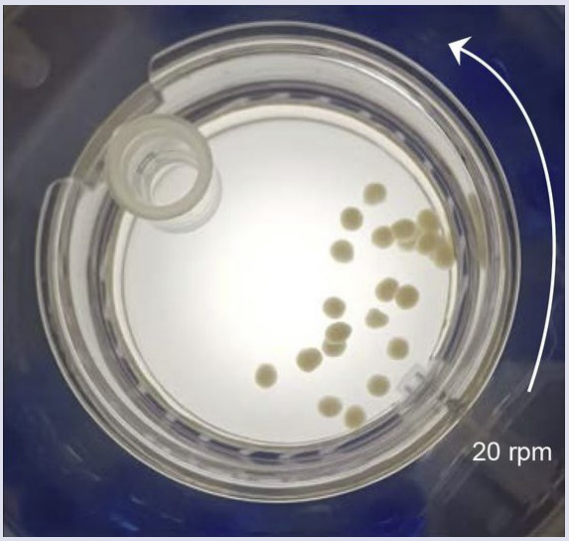
For their study, scientists generated organoids using hiPSCs derived from a human dermal fibroblast line. Cells were dissociated into single cell suspension, plated into ultra-low-binding plates for pre-aggregation under static growth for 5 days. Next, they were induced into neuronal lineage and on day 7 embedded in Matrigel, still grown under static conditions. To further differentiate these organoids in a system that supports long term culture conditions, continued maturation from day 10 to day 30 was conducted using the CelVivo ClinoStar (24 organoids per reactor at 20 RPM).

Organoids were grown till they reached 2-3 mm in size (see Figure 3).

After culture for 30 days, organoids were exposed to either the original ancestral isolate, BA.1 or BA.5 for a total of 4 days. Post treatment, scientists conducted immunohistochemistry using an anti-spike antibody, and observed the largest population of infected cells present in organoids after exposure to the BA.5 isolate (see Figure 3). Similarly, supernatant collected from the culture showed the highest viral titers present after exposure to BA.5 for all 4 days of treatment. Finally, analysis of RNA-Seq data indicated that infection with the BA.5 isolate resulted in a significant increase in the number of differentially expressed genes. Some of the changes recorded were active components of various pathogenic, infection, and inflammation inducing pathways, while other annotations were associated with neuropathology.

These findings were confirmed in a mouse model of severe COVID-19 symptoms, K18-hACE2. Scientists used this mouse strain to show that BA.5 infection leads to increased neurovirulence, encephalitis, and mortality compared to the older BA.1 variant.

Scan to read the full publication



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05.
Protocol

Omicron BA.5 infects human brain organoids and is neuroinvasive and lethal in K18-hACE2 mice

Romal Stewart, SevannahA. Ellis, Kexin Yan, Troy Dumenil, Bing Tang, Wilson Nguyen, Cameron Bishop, Thibaut Larcher, Rhys Parry, Robert K. P. Sullivan, Mary Lor, Alexander A. Khromykh, Frédéric A. Meunier, Daniel J. Rawle, Andreas Suhrbier

Doi: https://doi.org/10.1101/2022.12.22.521696

PROTOCOL

On day 0 of organoid culture, hiPSCs (less than passage 50) were dissociated with StemPro Accutase (Thermo Fisher Scientific) to generate a cell suspension.

5000 cells were plated in each well of an ultra-low-binding 96-well plate (Corning) in StemFlex media supplemented with 10 µM ROCK inhibitor Y-27632 (Stemcell technologies).

From days 1-5, daily media changes were carried out with StemFlex medium supplemented with 2 µM Dorsomorphine (Abcam) and 10 µM SB-431542 (Stemcell technologies).

CelVivo Clinostar incubator. (Size: 2-3 mm)

On day 5, the medium was replaced with a Neuroinduction medium consisting of DMEM/F12 (Thermo Fisher Scientific), 1% N2 Supplement (Thermo Fisher Scientific), 10 µg/ml Heparin (Stem cell technologies), 1% Penicillin/Streptomycin (Thermo Fisher Scientific), 1% Non-essential Amino Acids (Thermo Fisher Scientific), 1% Glutamax (Thermo Fisher Scientific) and 10 ng/ml FGF2 (Stemcell Technologies).

On day 7, organoids were embedded in Matrigel (Corning), transferred to an ultra-low-binding 24-well plate (Corning) (one organoid per well), and continued to grow in Neuroinduction medium for three more days.

On day 10, organoids were supplemented with differentiation medium, consisting of Neurobasal medium, 1% N2, 2% B27 supplements (Thermo Fisher Scientific), 0.5% Penicillin/Streptomycin, 1% Glutamax, 1% Non-essential Amino Acids, 50 µM of 2-Mercaptoenthanol (Merck), 2.5 µg/ml Insulin (Merck), 1% Knockout Serum Replacement (Thermo Fisher Scientific), 10ng/ml FGF2, 1 µM CHIR99021 (Stemcell Technologies) and placed in a CelVivo Clinostar incubator (Invitro Technologies) (24 organoids per ClinoReactor) spinning at 20 rpm. All media changes from 10 days onwards were performed every other day.

This white paper was written and reviewed by:

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