2024

CERO REPORT

JULY - OCTOBER

SEVENICH LAB
SUPPORTED BY OLS

CERO 3D Incubator and Bioreactor

Establishing Murine aNSC-Based Protocols Using the CERO Bioreactor

Project Update

This project marks the first use of the CERO bioreactor for culturing murine aNSCs, developing cerebral organoids, and brain cancer assembloids. While CERO has been tested with human stem cells, we evaluated its performance with murine aNSCs, comparing it to traditional well-plate methods. Results were analyzed for scalability, growth dynamics, and long-term culture stability.

S SCALABILITY

Handling larger cell cultures with less manual work and greater consistency.

G GROWTH DYNAMICS

Achieving more controlled growth and less frequent splitting and manual labor.

L LONG-TERM CULTURE

Sustaining organoid growth over extended periods with consistent shape and minimal intervention.

Reproducible 3R (Replace, Reduce, Refine) 3D culture systems are becoming increasingly crucial, as researchers seek reliable and consistent models. Traditional methods often lack the reproducibility required for advanced studies. In collaboration with OLS, we demonstrate the **value of the CERO bioreactor** in optimizing murine aNSC models for more robust, scalable, and reproducible results. **— Sevenich LAB**

Key Findings

The following highlights showcase the superior performance of the CERO bioreactor in culturing murine adult neural stem cells (aNSCs) and cerebral organoids. These key facts demonstrate its advantages in terms of scalability, efficiency, and long-term culture stability, significantly improving traditional culture methods.

20x

Higher aNSCs culture capacity

Supports up to 50 million cells per tube, compared to traditional well-plate methods.

5 days

For initial neurosphere formation

with slow controlled growth and minimal clumping.

2-4x

Less frequent aNSCs splitting

Continuous movement delays early neurosphere formation, reducing the need for frequent passaging.

50 %

Reduction in manual labor

Fewer media changes and less hands-on time compared to well-plate cultures.

> 200

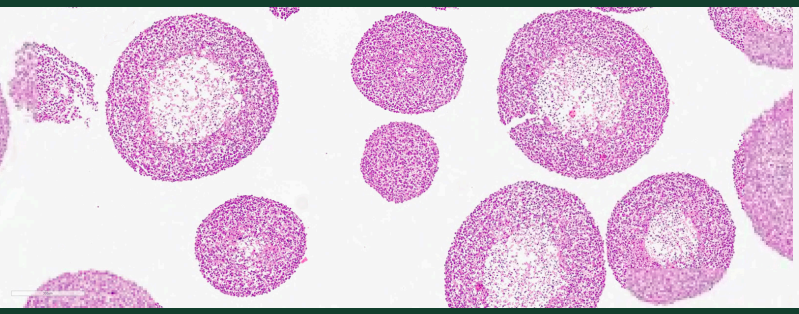
Cerebral organoids

grown per CERO tube, ensuring large-scale organoid production.

> 5

Months

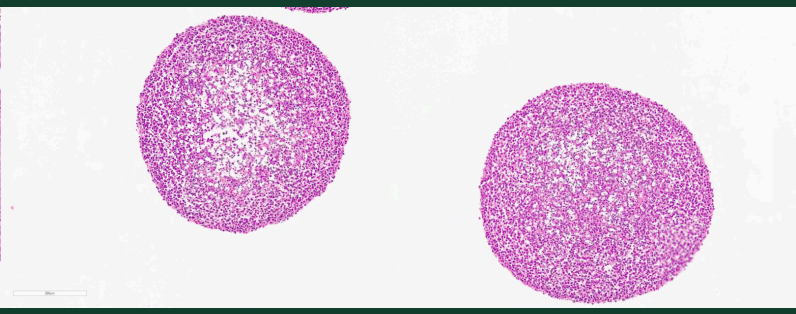
of stable organoid culture with consistent shape and size.



300 µM

Cerebral organoid after 3-month cultivation (6-well suspension plate/orbital shaker)

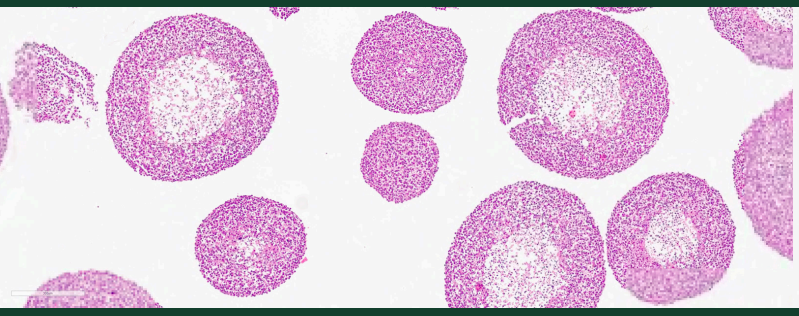
Characterized by uneven size distribution, oval shapes, and apoptotic cores.



300 µM

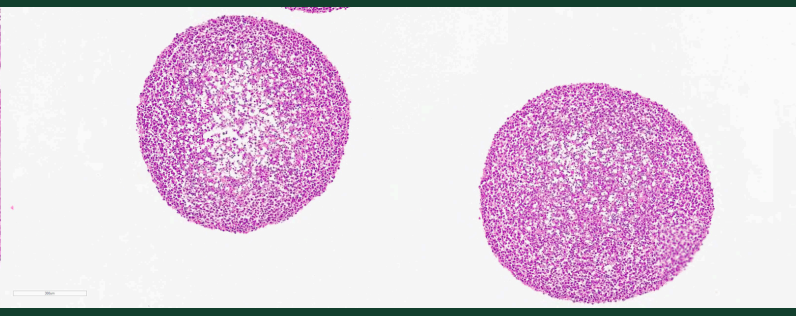
Cerebral organoid after 5-month cultivation (CERO 3D Incubator and Bioreactor)

Characterized by even size distribution, round shapes, and proliferating cores.



300 µM

Cerebral organoid after 3-month cultivation
(6-well suspension plate/orbital shaker)



300 µM

Cerebral organoid after 5-month cultivation
(CERO 3D Incubator and Bioreactor)

S SCALABILITY

6-Well Suspension Plate/Orbital Shaker:

- **Limited Capacity:** Supports ~10-30 organoids per well, but as they grow, organoids tend to touch the bottom, impacting growth.
- **Size Variability:** Organoids larger than 800 µm often develop uneven sizes and doughnut shapes due to space constraints and inconsistent mixing.

CERO 3D Incubator and Bioreactor:

- **High Capacity:** Cultures 200+ organoids per tube, significantly increasing throughput.
- **No Fusion:** Constant movement prevents fusion of organoids, maintaining controlled growth.
- **More Efficient:** Larger media volume reduces the need for frequent media changes, saving resources and time.

G GROWTH DYNAMICS

6-Well Suspension Plate/Orbital Shaker:

- **Uneven Growth:** Organoids show size variation and tend to flatten or form doughnut shapes once they grow over 800 µm.
- **Bottom Contact:** Organoids settle on the well bottom, disrupting uniform growth.

CERO 3D Incubator and Bioreactor:

- **Controlled Growth:** Slower initial formation allows for even, round organoids, preventing early fusion.
- **Consistent Suspension:** Organoids stay in suspension, avoiding bottom contact and ensuring uniform growth and nutrient access.

L LONG-TERM CULTURE

6-Well Suspension Plate/Orbital Shaker:

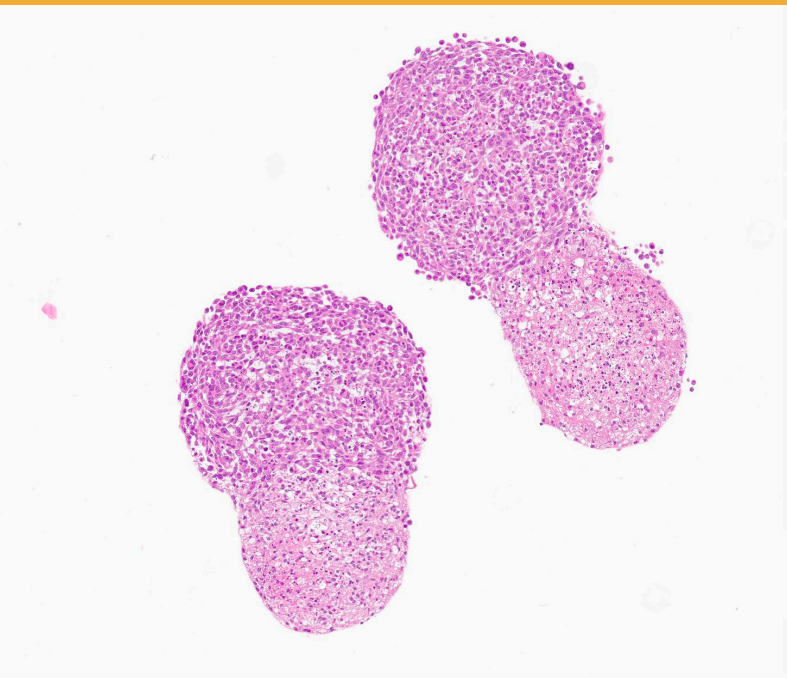
- **Viability Declines After 3 Months:** Larger organoids develop apoptotic cores and require replacement.

CERO 3D Incubator and Bioreactor:

- **Stable for 5+ Months:** Organoids remain viable with proliferating cores, round shapes, supporting extended culture without renewal.

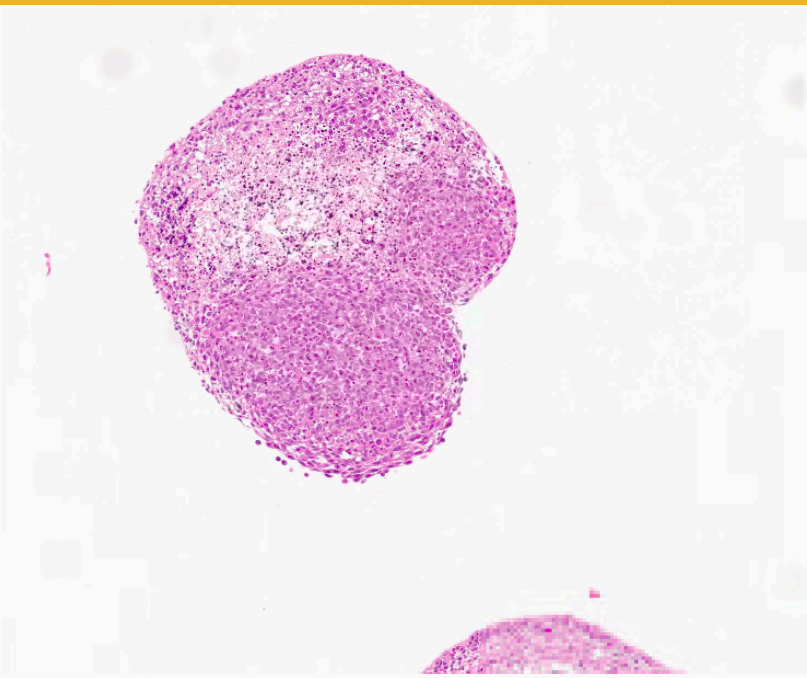
The CERO bioreactor offers 1.6x longer culturing time, supports 15x more organoids per unit, ensures even size up to 2 mm, and requires 50% less manual work compared to traditional 6-well suspension plates.

Glioma assembloid
(6-well suspension plate/orbital shaker)



300 µM

Glioma assembloid
(CERO 3D Incubator and Bioreactor)



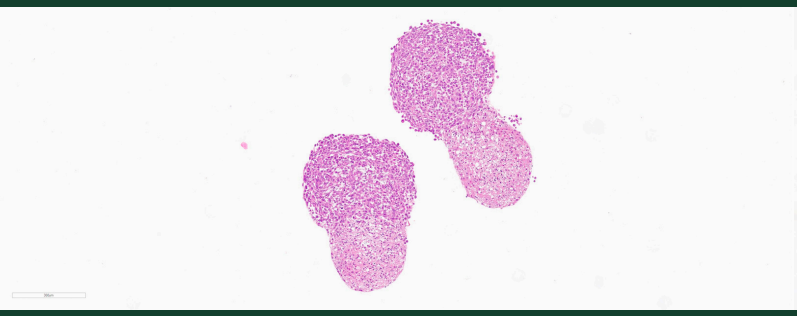
300 µM

Revolutionizing Assembloid Culture

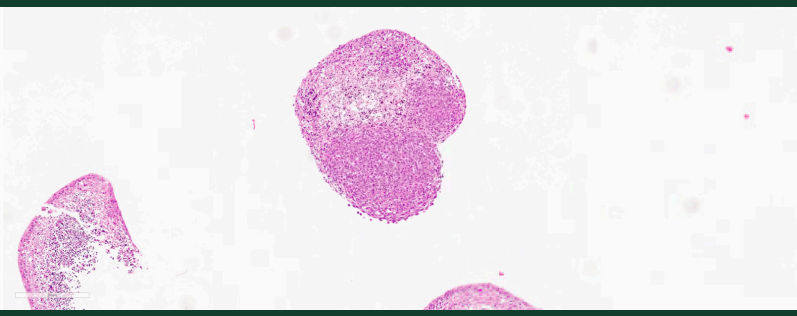
Cerebral Assembloids: A 3D Co-Culture Model

Cerebral assembloids, a co-culture of fused cerebral organoids and cancer spheroids, are an emerging 3R-compliant 3D model, offering an alternative to *in vivo* studies. Their key advantage lies in the ability to recapitulate both healthy and pathological tissue, closely mimicking conditions observed in cancer patients. However, despite their potential, assembloids have limitations. Both cerebral organoids and spheroids are spherical structures placed together, but their interaction sites are limited, with typically **less than 10% of their surface areas interconnected**. This does not represent the clinical scenario, where brain cancer is often fully surrounded by healthy brain tissue, creating more complex tumor microenvironment (TME) interactions.

In our **proof-of-concept study**, we demonstrate that the CERO bioreactor overcomes this limitation, producing **assembloid models with over 50% interconnected surface areas**, a significant improvement that better reflects the tumor-tissue interactions seen in clinical settings.



300 µM **Glioma assembloid**
(6-well suspension plate/orbital shaker)

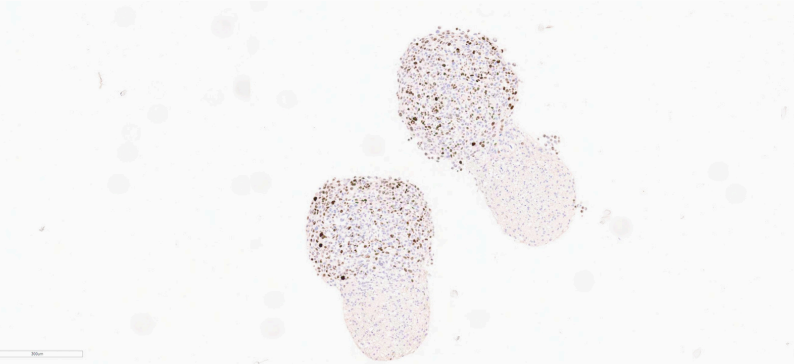


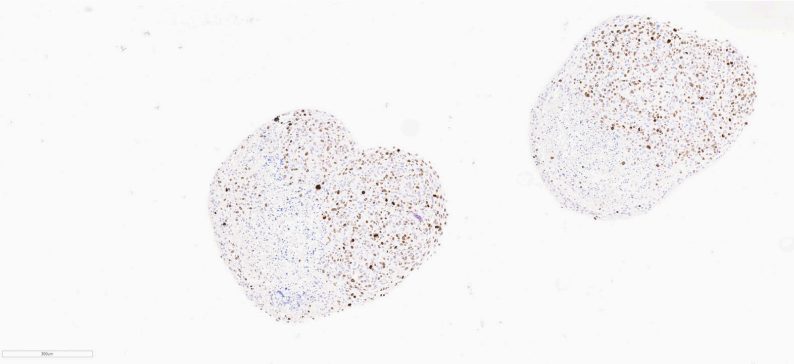
300 µM **Glioma assembloid**
(CERO 3D Incubator and Bioreactor)

P PROCEDURE SUMMARY

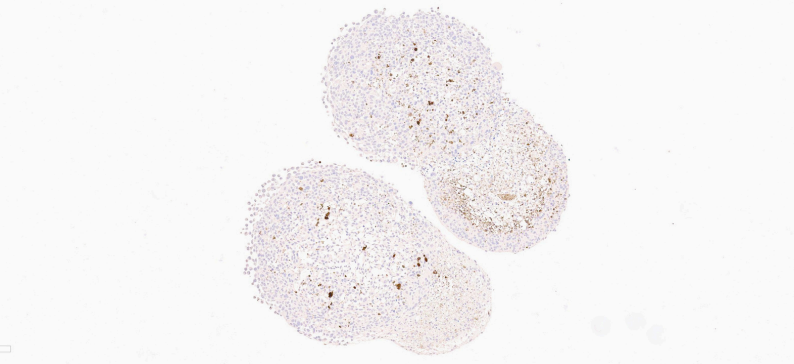
Previously differentiated cerebral organoids cultured were co-cultured with GL261 glioma spheroids (seeded at 10,000 cells per well in a 96-well ULA plate and pre-incubated for 3 days). Organoids and spheroids were placed together in a new 96-well ULA plate, allowing tight cell-cell connections to form over 72 hours. After 72 hours, half of the assembloids were transferred to a CERO tube, while the other half remained in the 96-well ULA plate. After an additional 48 hours (a total of 120 hours), assembloids were fixed, sectioned, and stained using H&E, Ki-67 and Cas-3 for analysis.

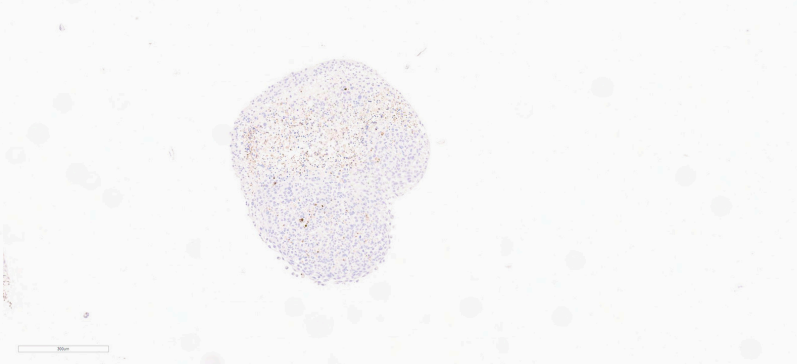
KI-67 PROLIFERATION MARKER





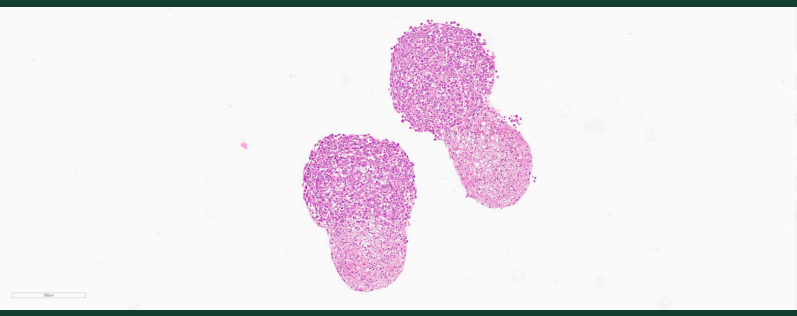
CAS-3 APOPTOSIS MARKER





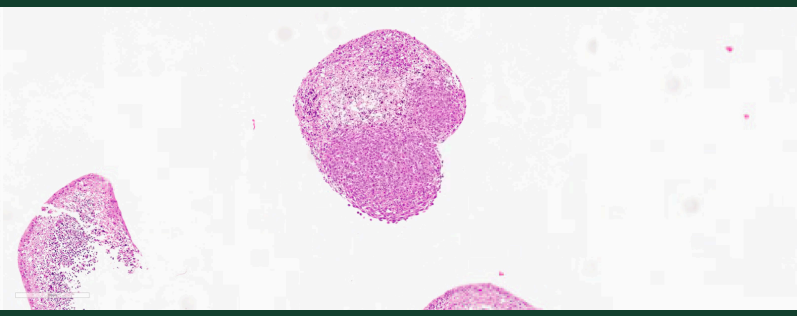
300 µM **Glioma assembloid**
(6-well suspension plate/orbital shaker)

300 µM **Glioma assembloid**
(CERO 3D Incubator and Bioreactor)



300 µM

Glioma assembloid
(6-well suspension plate/orbital shaker)



300 µM

Glioma assembloid
(CERO 3D Incubator and Bioreactor)

S SCALABILITY

96-Well ULA Plate:

- **Small-Scale Capacity:** Supports individual assembloid formation but is limited by the number of wells.

CERO 3D Incubator and Bioreactor:

- **Enhanced Complexity:** The primary strength lies in the greater model sophistication.
- **Multiple Assembloids:** Several assembloids can be cultured in a single CERO tube.

G GROWTH DYNAMICS

96-Well ULA Plate:

- **Limited Interaction:** Less than 10% surface interconnection between organoids and glioma spheroids, leading to weaker tumor-organoid interaction.
- **Decreased Proliferation:** Ki-67 staining shows reduced proliferation in the organoid part of the assembloid over prolonged culture.
- **Increased Apoptosis:** Cas-3 staining reveals higher levels of apoptosis in the organoid tissue compared to the CERO model.

CERO 3D Incubator and Bioreactor:

- **Improved Fusion:** Promotes over 50% surface interconnection between organoids and tumor spheroids.
- ***In Vivo* Mimicry:** Closely mimics tumor invasion, with H&E staining showing cerebral organoids growing around more than half of the tumor tissue.
- **Increased Proliferation:** Ki-67 staining reveals proliferation in both glioma and organoid tissues.
- **Enhanced Viability:** Cas-3 staining shows less apoptosis, indicating improved cell viability compared to the ULA plate.

L LONG-TERM CULTURE

96-Well ULA Plate:

- **Limited Interaction:** Organoid-tumor fusion remains weak, affecting the robustness of long-term studies.
- **Higher Apoptosis:** Increased apoptosis rates, as shown by Cas-3 staining, reduce the model's viability for extended research.

CERO 3D Incubator and Bioreactor:

- **Improved Fusion:** Enhanced organoid-tumor interaction supports better long-term culture.
- **Reduced Apoptosis:** Lower levels of apoptosis with proliferating cores in both glioma and organoid tissues create a more stable, long-lasting model.

The CERO bioreactor enhances assembloid culture by promoting greater organoid-tumor fusion, reducing apoptosis, and enabling more complex, long-term stable models with multiple assembloids in a single culture.

> **The CERO bioreactor has significantly boosted our productivity, reduced hands-on time, and opened exciting new avenues for research.**

Special Thank You

We are truly grateful to **Dr. Andreas Friese** and **Dr. Markus Uhrig** at OLS OMNI Life Science for giving us the opportunity to work with the CERO. Your support and specialist training have been invaluable and it has been a pleasure collaborating with both of you. We look forward to continuing our joint work, now focusing on brain slice cultivation.

Anna Wolfram, Vanessa Arnold & Prof. Dr. Lisa Sevenich