Immunocompetent Human Brain Organoids for Modeling Alzheimer's Disease

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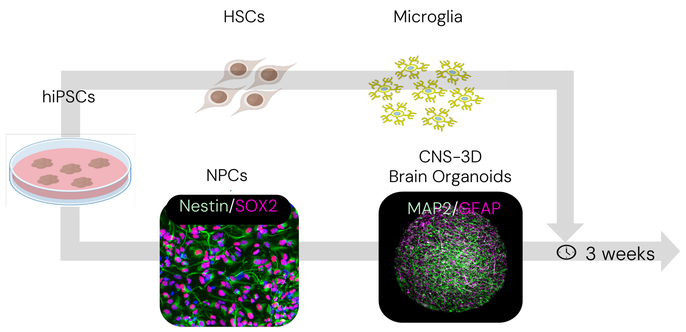
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Introduction

The unmet medical need associated with Alzheimer's disease (AD) and AD-related dementias (ADRD) is daunting. Despite tremendous public and private resources directed toward understanding AD pathology and developing drug candidates, progress has been frustratingly slow and there are few approved treatments successfully providing disease modifications. Using human induced pluripotent derived stem cells (hiPSCs), 28bio has developed a scalable cortical organoid model (CNS-3D Inflammatory Organoids) that exhibits robust, reproducible, and clinically relevant functional phenotypes. While CNS-3D Brain Organoids exhibit the most highly represented cell types in the brain (neurons and glia), we further improved the technology by integrating microglia to enable an often-absent neuroinflammatory component critical to modeling Alzheimer's Disease.

Generation of Inflammatory Organoids

CNS-3D Inflammatory Organoids (CNS-3D + MG) were generated by seeding neural progenitor cells (NPCs) into ultra-low attachment plates wherein they self-organized into 3D structures of ~50% neurons and ~50% astrocytes. After 3 weeks of maturation, microglia, derived from hematopoietic stem cells (HSCs), were seeded on to the organoids and were given 3 weeks to fully integrate and mature.



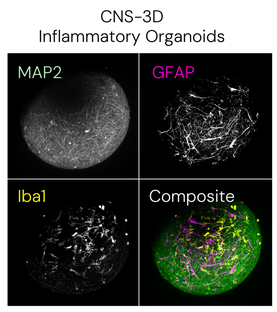


Figure 1 – Schematic of CNS-3D Inflammatory Organoid production. hiPSCs from a healthy donor[1] serve as the starting material for parallel production of standard CNS-3D Brain Organoids and microglia. For organoid production, iPSCs are first derived into Nestin/SOX2 positive NPCs, which upon aggregation in ULA plates, co-differentiate into neurons (MAP2) and astrocytes (GFAP). Microglia generation requires a 14-day protocol for HSC generation followed by a 24-day protocol for microglia production. After 3 weeks of organoid culture the iPSC-derived microglia were seeded at 3,000 microglia/organoid to create CNS-3D Inflammatory Organoids.

Microglia-incorporated organoids appear healthy and intact with little visual evidence of microglia incorporation aside from a smaller diameter, consistent with microglia's role in clearing cellular debris.

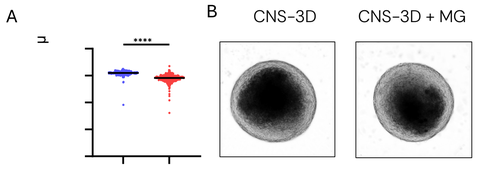
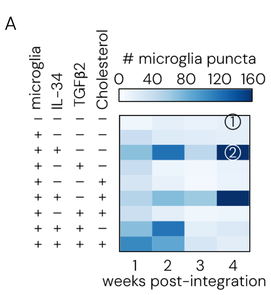
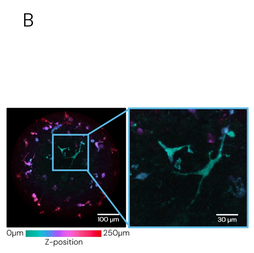


Figure 2 – Microglia incorporation decreases organoid size (A) Quantification of organoid diameter at 6 weeks of differentiation, 3 weeks after microglia incorporation. (B) Representative vehicle-treated organoids. Statistical significance assessed using Welch's t-test; **** p < 0.0001,.

Optimizing Co-culture of CNS-3D and Microglia

Maintaining microglia for long culture durations in a resting state is essential for establishing an inducible Alzheimer's disease model. We investigated the impact of media composition on microglia survival in co-culture with CNS-3D Brain Organoids[2,3]. Microglia were detected via Iba1 staining. Confocal imaging was used to quantify the number of microglia per organoid. Supplementing standard CNS-3D organoid media with IL-34 was sufficient to promote microglia survival and stable incorporation up to four weeks post-integration.





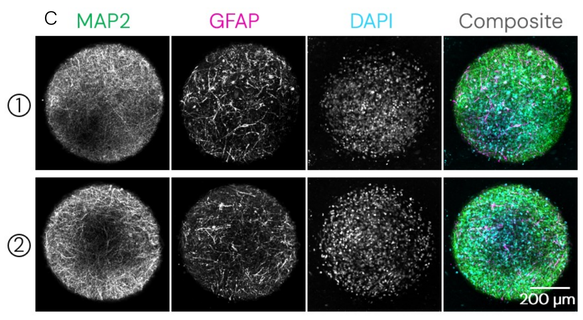


Figure 3 - IL-34 promotes long-term microglia survival and resting, ramified morphology without impacting neuronal and glial cell health. (A) Heatmap of microglia puncta detected as a function of time and media formulation. (B) Spatial color-coded image of Iba1 staining in organoids after 4 weeks showing resting ramified morphology of iPSC-derived microglia. (C) Max intensity projection of organoids grown with and without IL-34 showing consistent neuronal and astrocytic morphologies.

Cell-Type Specific Responses to Inflammatory Insults

CNS-3D Inflammatory Organoids demonstrate stimulus-dependent, cell-type-specific responses to inflammatory challenge. LPS primarily produces an Iba1-positive microglial response, while TNF-α drives a more GFAP-positive astrocytic activation pattern. This differential reactivity shows that the model can resolve distinct neuroinflammatory phenotypes across microglial and astrocytic populations.

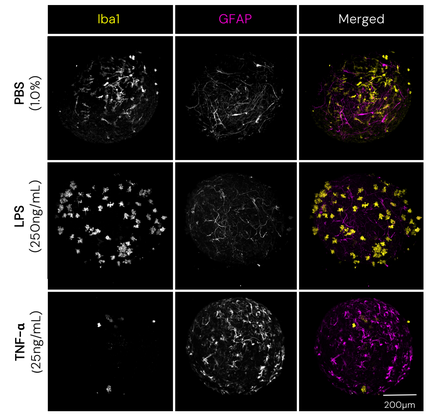


Figure 4 – Differential microglial and astrocytic responses to inflammatory insults in CNS-3D Inflammatory Organoids. CNS-3D Inflammatory Organoids were treated with vehicle, LPS, or TNF-α and assessed by immunofluorescence staining for Iba1-positive microglia and GFAP-positive astrocytes. LPS induced a pronounced microglial response, whereas TNF-α preferentially increased astrocytic activation, highlighting stimulus-specific inflammatory phenotypes within the 3D CNS organoid model.

Microglia Sensitize CNS-3D Organoids to Inflammatory Stimuli

Cytokine profiling after 24-hour exposure confirms that microglia incorporation sensitizes CNS-3D organoids to inflammatory challenge. Compared with CNS-3D Brain Organoids, CNS-3D Inflammatory Organoids produced markedly elevated cytokine levels following LPS exposure, including a 100-fold increase in IL-10, and >1000-fold increase in IL-6 in media supernatants. This enhanced response indicates that integrated microglia establish a more immunologically responsive CNS organoid environment, enabling robust detection of both pro-inflammatory and regulatory cytokine activity. The addition of microglia alone increased baseline levels of IL-1β and IL-10, further indicating that microglia are functionally interacting with the organoid environment. These data support the use of CNS-3D Inflammatory Organoids as a human-relevant platform for modeling neuroinflammatory signaling related to Alzheimer's Disease progression.

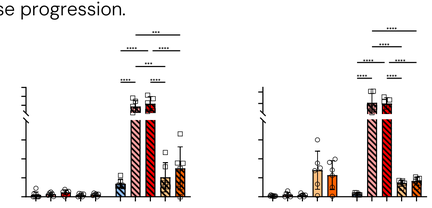


Figure 5 – Cytokine release profiling following LPS and TNF-α stimulation in CNS-3D and CNS-3D Inflammatory Organoids. Standard CNS-3D organoids and microglia-containing CNS-3D Inflammatory Organoids were exposed to inflammatory stimuli for 24 hours, and conditioned media were analyzed for IL-10, and IL-6 release. Data are shown across vehicle, LPS, and TNF-α treatment conditions. Statistical significance was assessed using two-way ANOVA with Tukey's multiple comparisons; * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

Amyloid-β Oligomer Induced Neurodegeneration

Inducing the CNS-3D Inflammatory Organoids with Amyloid-β shows oligomer-specific toxicity, observed as deterioration in organoid electrophysiological activity compared against other Aβ species.

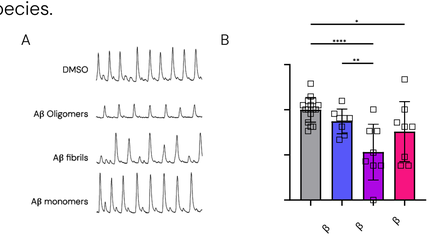


Figure 6 –Amyloid- β induced Functional Changes. CNS-3D Inflammatory Organoids were treated with 10µM Aβ oligomers, fibrils and monomers for 1 week. FLIPR traces (A) shows the visual changes to the organoid waveforms. Significant changes in Total Peak Frequency (B) quantified. Statistical significance was assessed using two-way ANOVA with Tukey's multiple comparisons; * p < 0.05, ** p < 0.01,, **** p < 0.0001.

Immunostaining reveals neuronal degradation and appreciable caspase activation following Aβ oligomer dosing.

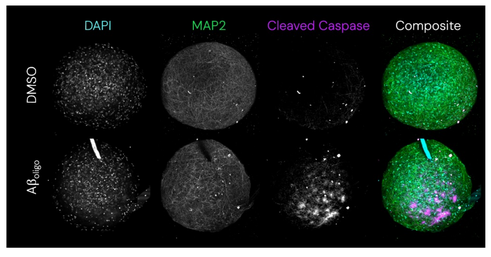


Figure 7 –Amyloid- β Oligomers Induced Neurodegeneration. Representative images of Aβ treated CNS-3D inflammatory organoids. Organoids were stained for DAPI (nuclei), MAP2 (neurons) and Cleaved Caspase 3 (apoptotic cells). Images are 800x800um.

Conclusions

- CNS-3D Inflammatory Organoids enable modeling of key neurodegenerative processes including neuroinflammation and amyloid- β induced toxicities.
- Early integration of iPSC-derived microglia into CNS-3D Brain organoids supports stable microglia incorporation, with IL-34 further enhancing long-term microglia survival.
- CNS-3D Inflammatory Organoids exhibit stimulus-specific neuroinflammatory responses, including distinct microglial and astrocytic responses to LPS and TNF-α.
- Microglia incorporation sensitizes CNS-3D organoids to inflammatory challenge, producing robust cytokine release following LPS exposure and demonstrating functional neuroimmune responsiveness.
- Amyloid-β specific toxicity is observed along both functional and image-based endpoints in CNS-3D Inflammatory Organoids.