Application Note
BrainZell x bit.bio

A human 3D neuroinflammation model using BrainZell organoids with integrated bit.bio ioMicroglia

MODEL
Human 3D neuroinflammation
BrainZell organoids integrated with bit.bio ioMicroglia.

ACTIVATION
LPS-induced response
Inflammatory gene, cytokine, and protein signatures.

MODULATION
Dexamethasone suppression
Pharmacologically reversible inflammatory response.

01 Key message

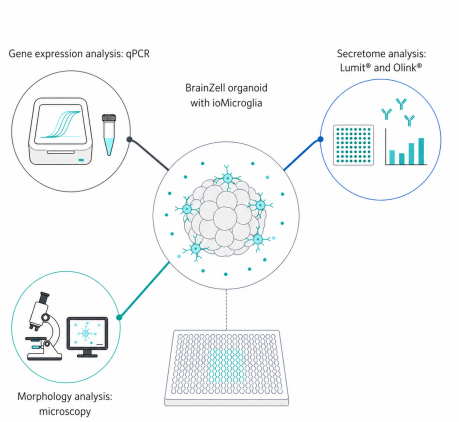
A human 3D neuroinflammation model was established by integrating BrainZell organoids with bit.bio ioMicroglia. The model captures key neuroimmune features, including microglial marker expression, cytokine release, and dexamethasone-sensitive modulation of inflammatory responses. The platform supports studies of neuroinflammatory mechanisms and detection of drug-induced immunomodulatory effects.

02 Introduction

Neuroinflammation is a key feature of neurological and neurodevelopmental disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, autism spectrum disorder, and traumatic brain injury. Microglia, the resident immune cells of the central nervous system (CNS), mediate inflammatory responses and contribute to brain homeostasis by regulating cell numbers, neural circuitry, and synaptic connectivity [1, 2].

Human iPSC-derived in vitro brain organoid models provide the closest fidelity to the human brain tissue. However, they often lack microglia, limiting their ability to capture neuroimmune interactions relevant for deciphering disease biology, therapeutic response, and drug-induced CNS inflammation [3, 4]. Therefore, human-relevant 3D models incorporating functional microglia are needed to generate more physiologically relevant data and improve drug discovery outcomes.

BrainZell organoids provide a reproducible human 3D neural environment comprising multiple neuronal subtypes and astrocytes. To enhance their neuroimmune relevance, bit.bio ioMicroglia (cat. no. io1021) were integrated into BrainZell organoids. Inflammatory responsiveness was assessed using lipopolysaccharide (LPS) and pharmacological modulation was evaluated using dexamethasone [5, 6, 7]. This application note demonstrates microglial integration, inflammatory activation, and anti-inflammatory modulation in a human 3D neural context.



Graphical overview. Organoid-microglia 3D models were generated by integration of BrainZell organoids with ioMicroglia. After adding microglia, organoids were further cultured to allow establishment of fully integrated tissue before downstream analysis.

03 Results

BrainZell organoids successfully integrated ioMicroglia

The presence of integrated ioMicroglia in BrainZell organoids was assessed using the pan-microglial marker IBA1 [8, 9]. Immunohistochemistry showed IBA1-positive cells distributed throughout the organoid structure with microglia-like morphology (Figure 1).

To quantify microglial abundance, qPCR was performed across multiple microglial seeding densities. *IBA1* expression increased with the proportion of microglia added (Figure 2).

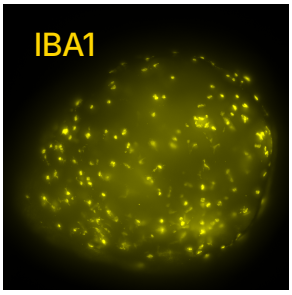


Figure 1. IBA1-positive microglia in BrainZell organoids. IBA1+ cells were detected within the organoid structure.

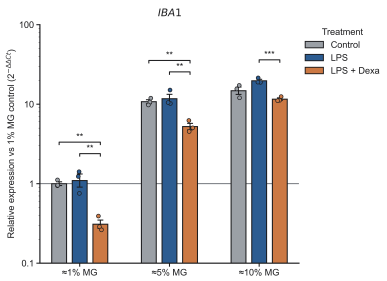


Figure 2. *IBA1* gene expression in BrainZell organoids incorporating ioMicroglia. Relative *IBA1* expression was measured by qPCR and normalized to the 1% microglia condition. Data are presented as mean ± SEM from independent biological replicates. Statistics were performed using Welch's t-test with Holm correction.

LPS-induced neuroinflammatory response in BrainZell organoids integrated with ioMicroglia

Inflammatory activation was evaluated using TNF-α gene expression and TNF-α and IL-6 protein secretion into the culture medium. LPS increased *TNF-α* gene expression, with the strongest response in organoids containing 5% and 10% microglia. Co-treatment with dexamethasone attenuated the LPS-induced *TNF-α* response, demonstrating pharmacological modulation of inflammatory activation (Figure 3).

At the protein level, LPS induced secretion of TNF-α and IL-6 in organoid-microglia co-cultures (Figure 4A-B, Lumit Promega W6050, W6030), confirming functional inflammatory responsiveness. TNF-α and IL-6 responses were strongest in conditions with higher microglial proportions, supporting the evidence for microglia-dependent inflammatory output. Dexamethasone reduced both LPS-induced TNF-α and IL-6 secretion, confirming that the response is sensitive to anti-inflammatory modulation. Together, these data show that the BrainZell organoid-ioMicroglia model can detect both inflammatory activation and pharmacological suppression in a human 3D neural context.

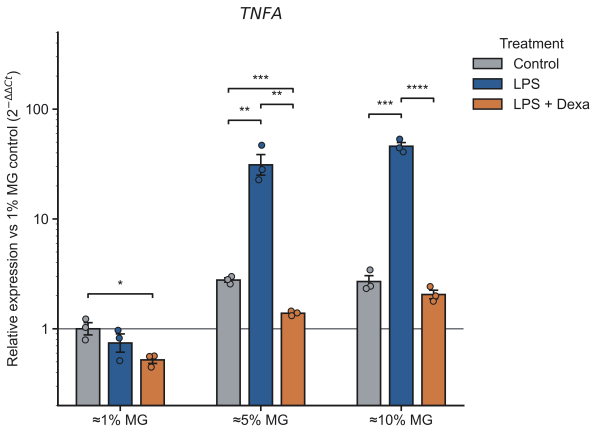


Figure 3. *TNF-α* gene expression in BrainZell organoid-ioMicroglia co-cultures. LPS increased *TNF-α* expression, and dexamethasone attenuated this response. Statistical comparisons were performed using Welch's t-test with Holm correction.

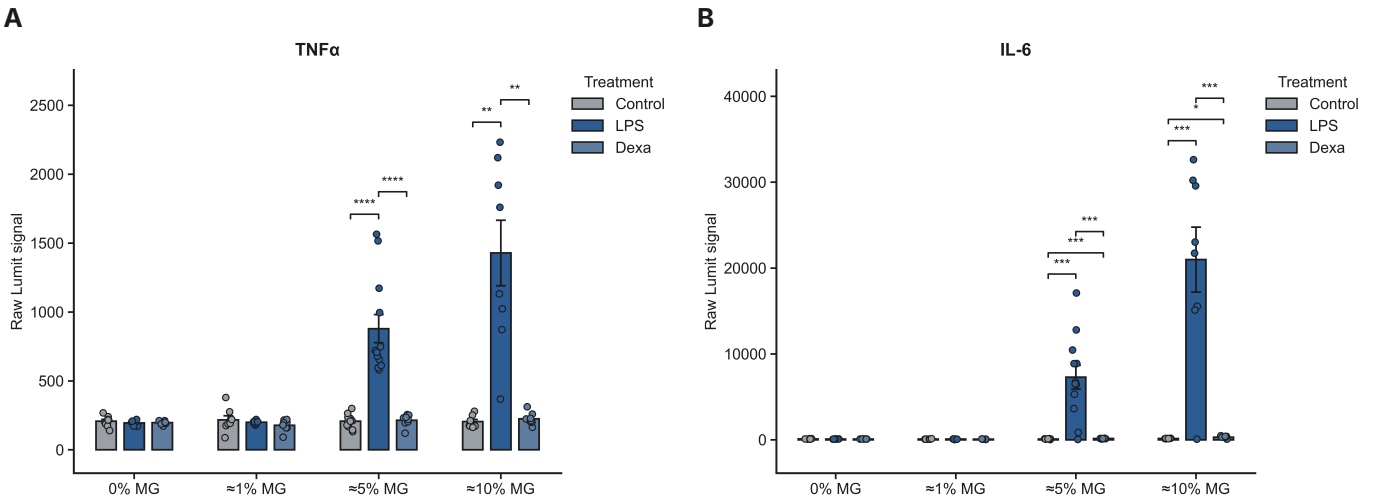


Figure 4. Cytokine secretion in BrainZell organoid-ioMicroglia co-cultures. Levels of TNF-α (4A) and IL-6 (4B) were measured in conditioned media using Lumit immunoassays. Data are presented as mean ± SEM from independent biological replicates. Statistics were performed using Welch's t-test with Holm correction.

To further characterize the inflammatory response, changes in secreted proteins following LPS stimulation and dexamethasone co-treatment were assessed using Olink inflammation and cytokine panels. Molecular Signatures Database (MSigDB) hallmark enrichment analysis identified strong enrichment of inflammation-associated pathways after LPS stimulation, including TNF-α signaling via NF-kB, inflammatory response, and IL-6/JAK/STAT3 signaling (Figure 5A). These pathways were, in turn, reduced by dexamethasone treatment, showing suppression of key components of the LPS-induced inflammatory program. Individual protein signatures contributing to the MSigDB enrichment included classical pro-inflammatory response proteins such as CCL20, IL1A, IL6, and LIF, which were upregulated by LPS and downregulated following LPS + dexamethasone treatment. In contrast, growth factors such as AREG and FGF2 showed a more partial or resistant response. (Figure 5B). Together, these data support the gene expression and cytokine release findings and provide a broader systems-level overview of neuroimmune activation and pharmacological suppression in the BrainZell organoid-ioMicroglia model.

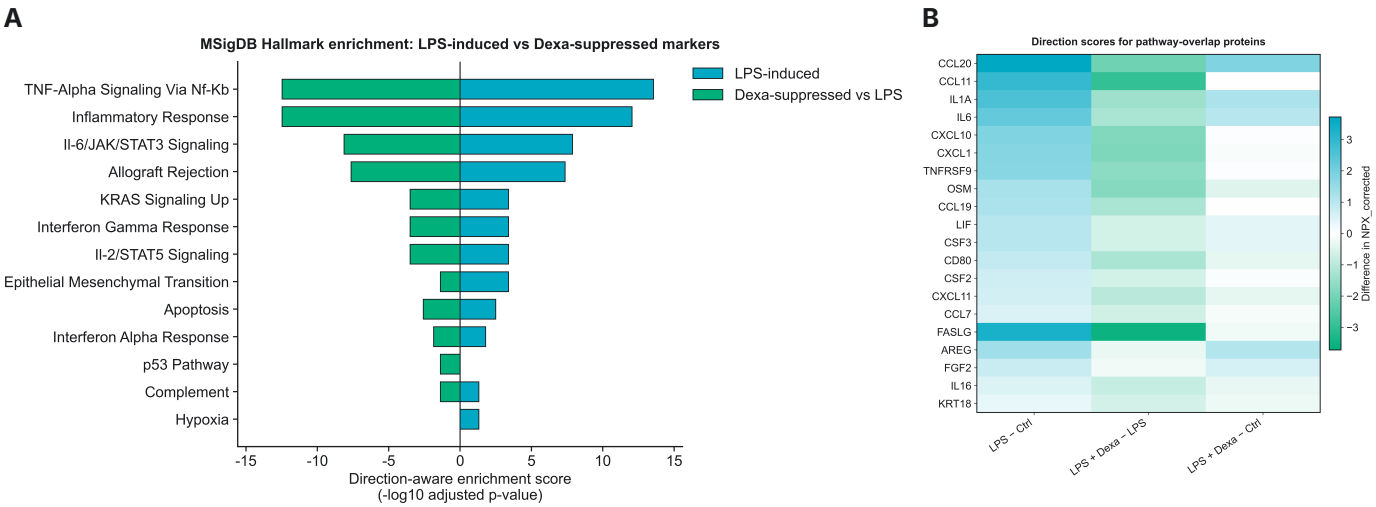


Figure 5. Protein profiling and pathway enrichment of inflammatory signatures. Olink analysis of conditioned media from Control, LPS, and LPS + Dexamethasone conditions. (A) MSigDB Hallmark enrichment showing activation of TNFA, IL-6/STAT3, and Interferon pathways. (B) Heatmap of differentially expressed proteins.

04 Clinical and translational relevance

Neuroinflammation contributes to multiple CNS disorders, yet many preclinical in vitro models lack microglia or do not capture inflammatory responses in a human 3D neural context. The BrainZell organoid-ioMicroglia model addresses this gap by enabling assessment of microglial identity, inflammatory activation, cytokine secretion, and anti-inflammatory suppression in one platform. Potential applications include studying neuroinflammatory mechanisms, screening anti-inflammatory or immunomodulatory compounds, evaluating CNS-targeting therapeutics, and supporting neurosafety assessment of modalities with potential inflammatory liability.

05 Discussion

Integration of BrainZell organoids with ioMicroglia establishes a robust human 3D neuroinflammation model. Microscopy and qPCR analysis of IBA1 confirmed microglial incorporation within the 3D neural environment, while functional validation with LPS and dexamethasone demonstrated a pharmacologically modifiable inflammatory response. This platform provides a human-relevant system for:

- **Neurodegenerative and neuropsychiatric disease modeling:** investigating microglia-mediated mechanisms in Alzheimer's disease, schizophrenia, autism spectrum disorder, and related conditions. Future avenues could include investigating the effects of adding ioMicroglia with disease-relevant genotypes such as APOE ε4/ε4.
- **Neuropharmacology and drug screening:** evaluating anti-inflammatory or immunomodulatory compounds in a predictive human-derived environment.
- **Neurosafety assessment:** identifying potential CNS inflammatory liabilities for biologics, gene therapies, antibodies, and other therapeutic modalities.

The platform supports multiple analytical workflows. Lumit immunoassays enable rapid protein-based readouts of TNF-α and IL-6, while Olink profiling provides a broader systems-level overview of inflammatory pathway activation and suppression. Together, these tools offer a scalable framework for translational neuroinflammation research and therapeutic development.

06 References

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