Summary & Impact

- Improved in vitro Alzheimer’s disease (AD) models may lead to greater insights into pathophysiology and potential treatments.
- Here we present several low-cell models used to study AD and developed for the use on the IncuCyte® Live-Cell Analysis System.
- Healthy and patient derived neuron-propagating cells were compared using 2D & 3D applications. The effect of Tau aggregation and inhibition of phosphorylation (via Okadaic acid, OA) in cell health, neurite outgrowth and neuronal activity was studied in primary cortical and cell line models using immunohistochemical and fluorescence imaging.
- Phagocytosis by microglia of p-tau181 labeled Amyloid beta (Aβ) peptide was assessed using cell line and human samples to investigate immune interactions.

This study shows that long term monitoring with multiple readouts from advanced cellular models, has the potential to deliver greater biological insight into neurodegenerative disorders, contributing to drug discovery.

Patient-derived AD iPSCs yielded lower neurite processes than healthy controls.

Validation of spherical size and growth potential; AD iPSC-derived neurons (K562 line) were seeded as described at densities of 30,000–50,000 cells/well. 

As expected, spherical size increased proportionally with seeding density.

To further evaluate growth potential the spherical size was normalized to 3% Spherical growth was inversely dependent, with the greatest growth rates observed for smaller cell lines.

To further evaluate growth potential the spheroid size was measured at 6 d. Spheroid development was quantified using NeuroPrime platform for up to 15 d. 3D sphere growth, differential size was measured using brightfield analysis for 6–15 s.

3D sphere growth: Development of spheroids was quantified using NeuroPrime system (Sartorius) for up to 15 d. 3D sphere growth, differential size was measured using brightfield analysis for 6–15 s.

3D neurodevelopment: Matrigel (30 µg/ml) was added to 4 s. Spherical monolayer/microbiome neurites were observed for a further 9 d.

Aggregation of Tau peptide induced greater neurotoxicity in primary neurons

To further explore the disease-relevant toxicity induced by aggregated Tau peptide, cell death (40% ± 3 replicates) and microglial activation was performed.

Images show the active range (maximum fluorescence) over a complete scan (3 minutes). Calcium traces represent calcium fluxes detected in live cells. Images were taken in the IncuCyte® system (3 minute raster scans at 3 frames per second).

Once functional, mature networks had formed (14 d) cells were treated with either the AD related peptide Tau (aggregated, 300 µg/ml) or the protein phosphatase inhibitor OA (50 µg/ml).

Images show the action range (maximum fluorescence) over a complete scan (3 minutes). Cell lines represent calcium fluxes detected in live cells. Images were taken in the IncuCyte® system (3 minute raster scans at 3 frames per second).

Oka decreased neuronal outgrowth in differentiated SH-SY5Y cell lines expressing Navichrome-Orange (Sartorius), which was assessed using gradient serum starvation (0% to 10% serum) and sequential addition of atkin (10 µM) and followed by 0.01% DMSO (5%–0%–0%).

Cells were treated with OA (1 µM – 20 µM), in media containing Annexin V NIR (25 µg/ml) at day 14.

Kinetic quantification of neuronal outgrowth was performed using the NeuroTrack application. OA induced concentration-dependent inhibition of neurite outgrowth EC50 = 1.6 ± 0.5 replicates.

Kinetic quantification of phagocytosis by microglia

Engulfment of Aβ is dependent on cell type, peptide aggregation and concentration

Spheroids were generated from cell lines and aggregated Aβ peptide (100 µg/ml) and incubated at 37°C for 24 h.

Elevated cytokine levels observed in AD neurons + microglia IPCS co-cultures

- Microglial precursors cells (macrophages) were seeded into 96-well plates at 30,000 cells/well and differentiated to mature microglia for 2 weeks. Peptides were labelled using IncuCyte® p-tau181 Orange Cell Labelling Kit and aggregates were formed at 37°C for 3 h. Cell viability was assessed for adhered cells (50 ± 5 replicates).
- Phagocytosis was measured using the IncuCyte® p-tau181 Orange (Essen Bioscience) dye. Microglia were differentiated from human peripheral blood mononuclear cells and added to selected wells and differentiated. On day 28 supernatants were removed and microglia from each well were analysed using the p-tau181 Orange dye.
- Cytokine levels in CSF were determined using the Luminex xMAP technology.


tau & oka toxicity induced loss of neuronal activity

- Primary cortical neurons (NeuroBlue®, Sartorius) were seeded in 96-well plates at 20,000 cells/well and placed in the IncuCyte® for the duration of the studies. 8-day post-seeding, cells were treated with vehicle or aggregated Tau (500 nM, 24 h). Cells were assessed at 0 to 5 replicates.

- Quantification of neurite outgrowth and cell health (AssayChef) were performed.

- Aggregated Tau peptide induced concentration-dependent toxicity with a minimum fluorescence (85 ± 3 replicates) at 1 µM aggregation and an increase in cell death (85 ± 3 replicates).

- Non-aggregated only induced calcium (calcium release of neurite formation at 150 ± 20 µM).

- Cortical neurons and microglia (NeuroBlue®, Sartorius) were seeded as a co-culture (20,000 and 15,000 cells/well respectively) in 96-well coated-96-well plates. Neurons were infected with the genetically encoded calcium indicator NeuroBurst Orange (Sartorius) to monitor spontaneous neuronal activity over time through measuring calcium fluctuations. Images were taken in the IncuCyte® (3 minute raster scans at 3 frames per second).

- Once functional, mature networks had formed (15 µg/ml) cells were treated with either the AD-related peptide Tau (aggregated, 300 µg/ml) or the protein phosphatase inhibitor OA (50 µg/ml).

- Cytokine levels in CSF were determined using the Luminex xMAP technology.