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Simplifying Progress

# Improved Alzheimer's disease models using neuronal and microglial live-cell analysis in 2D and 3D

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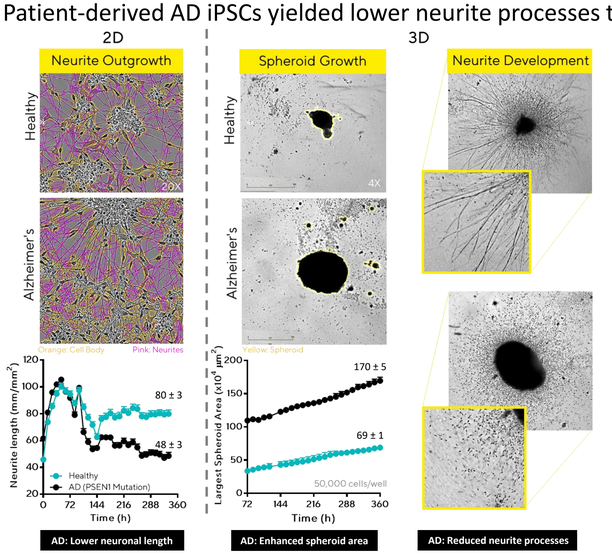
## Summary & Impact

- Improved *in-vitro* Alzheimer's disease (AD) models may lead to greater insight into pathology and potential treatments.
- Here we present several live-cell models used to study AD and developed for the use on the IncuCyte® Live-Cell Analysis System.
- Healthy and patient derived neuro-progenitor cells were compared using 2D & 3D applications. The effect of Tau aggregation and inhibition of phosphorylation (via Okadaic acid, OKA) in cell health, neurite outgrowth and neuronal activity was studied in primary cortical and cell line models using Annexin V NIR and NeuroBurst® reagents.
- Phagocytosis by microglia of pHrodo® labeled Amyloid-beta (Aβ) peptide was assessed using cell lines and hiPSCs to investigate immune interactions.
- This data shows that long-term monitoring, combined with multiple readouts from advanced cellular models, has the potential to deliver greater biological insight into neurological disorders, contributing to drug discovery.

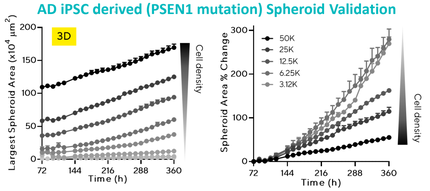
## Patient-derived AD iPSCs yielded lower neurite processes than healthy controls



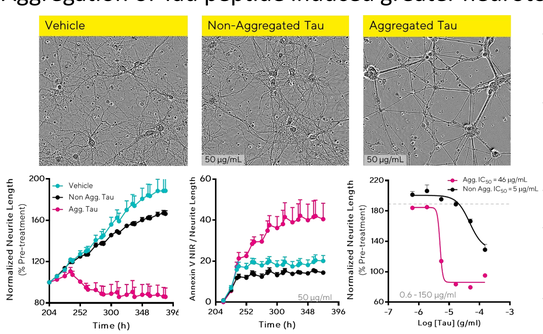

- Healthy and AD (PSEN1 mutation) derived iPSC neurons (Axol Bioscience).
- **2D plating:** ReadySet + SureBond coated 96-well plates at 25,000 cells/well
- **3D plating:** ULA 96-well plates at 50,000 cells/well and centrifuged (250 g; 10 mins). Spheroids were allowed to form for 3 d.
- Neuronal differentiation was induced as per supplier's protocol (supplements A+B).
- Cultures were placed in the IncuCyte® Live-Cell Analysis Platform for the duration of the studies.
- **2D neurite outgrowth**: development was quantified using NeuroTrack application (Sartorius) for up to 15d.
- **3D spheroid growth**: differential in size was quantified using Brightfield analysis for up to 15 d.
- **3D neurite development:** Matrigel® (2.25 mg/mL) was added at 6 d. Spheroid morphology/ neurite outgrowth from the spheroid body was observed for a further 9 d.

- Validation of spheroid size and growth potential; AD iPSC-derived neurones (Axol Bioscience) were seeded as described at densities of 390 – 50,000 cells/well.
- As expected, spheroid size increased proportionally with seeding density.
- To further evaluate growth potential the spheroid size was normalized to 72 h. Spheroid growth was inversely size-dependent, with the greatest growth rates observed for smaller spheroids.

### AD iPSC derived (PSEN1 mutation) Spheroid Validation

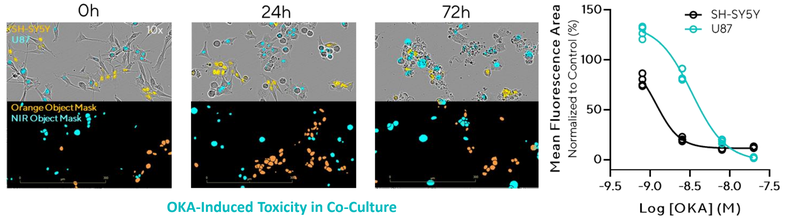



## Aggregation of Tau peptide induced greater neurotoxicity in primary neurons




- Primary rCortical neurons (NeuroPrime®, Sartorius) were seeded in PDL coated 96-well plates at 20,000 cells/well and placed in the IncuCyte® for the duration of the studies.
- 8 days post-seeding, cells were treated with solubilized or aggregated Tau (Heparin 4:1 ratio, 37°C with sporadic rotation for 5d) in media containing Annexin V NIR (0.25 µg/mL).
- Quantification of neurite outgrowth and cell health (Annexin V NIR) was performed.
- Aggregated Tau yielded a concentration-dependent decrease of neurite length (80 ± 5 vs. 166 ± 3 %, 2-3 replicates) and an increase in cell death (40 ± 8 vs. 14 ± 1 %, 3 replicates).
- Non-aggregated only yielded substantial inhibition of neurite formation at 150 µg/mL.

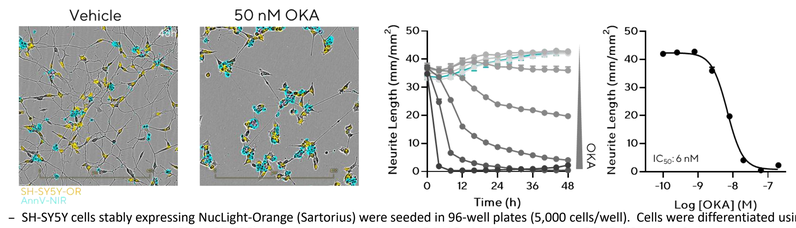
## Inhibiting phosphorylation selectively affected neuronal health & outgrowth




### OKA-Induced Toxicity in Co-Culture

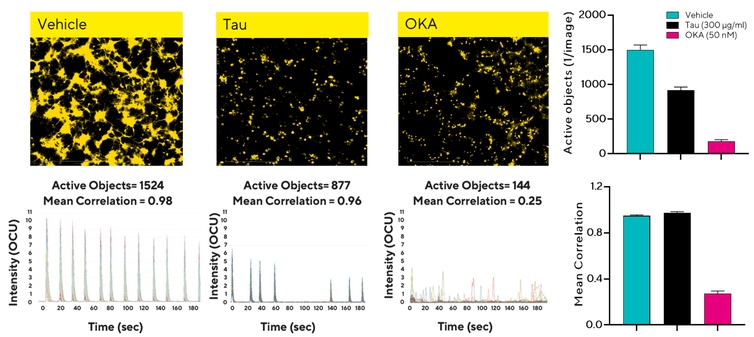
- Co-cultures of SH-SY5Y neuroblastoma and U87 astrocytic cells were stably transfected with NucLight-Orange or NucLight-NIR (Sartorius), respectively (10,000 cells/well of each cell type). 24h post-seeding, cells were treated with the selective PP1 & PP2A phosphorylation inhibitor OKA (6.25 – 200 nM) and phase and fluorescent images were acquired continuously using the IncuCyte® for 72h.
- Representative images following 12.5 nM OKA treatment. Concentration response curves show differential OKA-induced toxicity profiles for neurons and astrocytes with a suggested increase in potency for neuronal cells.

### OKA decreased neuronal outgrowth in differentiated SH-SY5Ys in Mono-Culture




- SH-SY5Y cells stably expressing NucLight-Orange (Sartorius) were seeded in 96-well plates (5,000 cells/well). Cells were differentiated using gradual serum starvation (10% to 0% FBS) and sequential addition of atRA (10 µM; 6 d) followed by BDNF (50 ng/mL; 6 d).
- Cells were treated with OKA (1.5 – 200 nM), in media containing Annexin V NIR (0.25 µg/ml) on day 14.
- Kinetic quantification of neuronal outgrowth was performed using the NeuroTrack application. OKA induced concentration-dependent inhibition of neurite outgrowth ($IC_{50}$ = 6 nM; 5 replicates).

## Tau & OKA toxicity induced loss of Neuronal Activity




- rCortical neurons and rAstrocytes (NeuroPrime®, Sartorius) were seeded as a co-culture (20,000 and 15,000 cells/well respectively) in PDL coated 96-well plates. Neurons were infected with the genetically-encoded calcium indicator NeuroBurst-Orange (Essen Bioscience, Sartorius) to monitor spontaneous neuronal activity over time through measuring calcium fluctuations. Images were taken in the IncuCyte® (3 minute 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 OKA (50 nM).
- Images show the active range (maximum – minimum fluorescence) over a complete scan (3 minutes). Calcium traces represent calcium fluctuation of all active objects within the field of view. Bar graphs provide the quantification of the number of active objects (1/image) and the correlation (connectivity).
- Compared to vehicle, Tau treatment decreased the number of active nodes (1407 ± 94 vs. 920 ± 43 objects/image) and their mean intensity (13.7 ± 1.7 vs. 7.2 ± 3.6 OCU), whilst not affecting correlation (0.95 ± 0.01 vs. 0.97 ± 0.01; 2 replicates).
- OKA decreased the number of active nodes (180 ± 24 objects/image), mean intensity (3.8 ± 0.3 OCU) as well as correlation (0.27 ± 0.02; 6 replicates) compared to vehicle.

## IncuCyte® System for continuous live-cell analysis

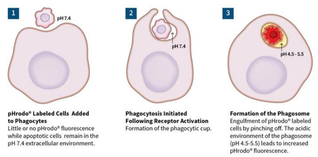
**IncuCyte® Live-Cell Analysis System**
A fully automated phase contrast and multi-colour fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

**IncuCyte® Software**
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

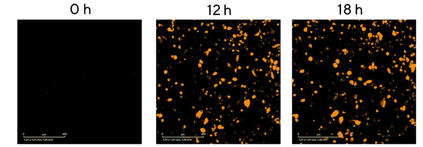
**IncuCyte® Reagents and Consumables**
A suite of non-perturbing cell labelling and reporter reagents.

## Studying Neuro-Inflammation: iPSC microglia engulf aggregated Aß peptide

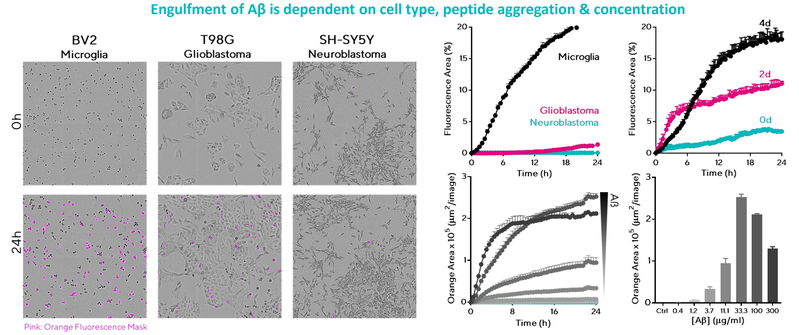
### Kinetic quantification of phagocytosis by microglia




### Phagocytosis of aggregated Aß (100 µg/ml)

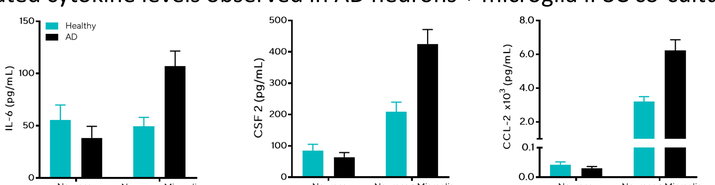



### Engulfment of Aß is dependent on cell type, peptide aggregation & concentration




- hiPSC microglial precursor cells (Axol Bioscience) were seeded into 96-well plates at 30,000 cells/well and differentiated to mature microglia for 2 weeks. Peptides were labelled using IncuCyte® pHrodo® Orange Cell Labelling Kit and aggregates were formed at 37˚C for 48h prior to assay and added to cells (0.4 – 300 µg/mL). Phase and fluorescent images were acquired in IncuCyte® for 36h.
- Representative images comparing Aβ uptake and differences in morphology between cell lines at 24h.
- Kinetic graphs display the cell-type, aggregation and concentration-dependent response to pHrodo® labeled Aβ peptide over time. Where different cell types were compared fluorescence area was normalized to phase area to account for variations in cell morphology and number. Bar chart displays orange area for each concentration at 24h.

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




- Healthy and AD (PSEN1 Mutation) derived iPSC neurons were plated and differentiated as previously described (2D Characterization; figure 1).
- On day 19, iPSC-derived monocytes (Axol Bioscience) were added to selected wells and differentiated. On day 30 supernatants were removed and neuroinflammation-related cytokine levels were analysed using the iQue3® flow cytometer.
- Preliminary data suggests that when neurons are co-cultured with microglia the levels of inflammatory cytokines IL-6 (107 ± 15 pg/ml), CSF-2 (425 ± 46 pg/ml), and CCL-2 (6300 ± 227 pg/ml) were consistently higher for the disease phenotype compared to healthy controls (50 ± 9 pg/ml, 209 ± 31 pg/ml, and 3190 ± 300 pg/ml, respectively; 6 replicates).

All data contained in this poster is expressed as Mean ± SEM