Visualising Endosomal Material Transfer using Aco-Dyes™: Tracking Disease Progression with 3D Organoid Cultures

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This application note is based on a preprint publication "Molecular and Structural Characterization Reveals Divergent Extracellular Vesicle Profiles Between Wild Type and Alzheimer's Disease Cerebrocortical Organoids" by Balistreri *et al.*[1].

Introduction

Extracellular vesicles (EVs) are key mediators of intercellular communication. EVs can serve as biomarkers for detecting and monitoring the progression of diseases such as cancer and neurodegeneration. However, the detection of these nano-sized vesicles is difficult, especially under biological conditions. Generally, fluorescent probes are used to tag EVs for visualising, tracking, and quantifying EVs in situ.

Conjugated oligoelectrolytes (COEs), commercialised as Acoerela™ Dyes (Aco-Dyes™), are fluorescent probes that intercalate into lipid bilayers[2]. Their unique mechanism enables robust and consistent labelling of both cells and EVs, facilitating their visualisation and characterisation across a range of fluorescence-based analytical platforms.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that accounts for more than 60% of dementia cases worldwide[3]. Despite the huge research focus on AD in the past few decades, the success rate of clinical translation is low. Conventional 2D cell cultures fail to mimic the complexity of the brain while animal studies fail to recapitulate human-specific biology. The advancements in human stem cell-derived 3D cultures like organoids have provided researchers with a physiologically relevant, 3D human model to understand the complex biology of diseases like AD[4].

In this application note, Aco-650-labelled AD organoid was co-cultured with an unstained wildtype (WT) organoid. The visualisation and detection of the transfer of endosomal materials from the diseased model to the WT cerebrocortical organoid was performed using confocal microscopy (Figure 1). The detection of Aco-650 fluorescent signals in the originally unstained

WT organoid validates inter-organoid communication, which may provide an explanation for the pathogenesis of AD. With a better understanding of the mechanisms of disease progression, novel treatment options can be developed.

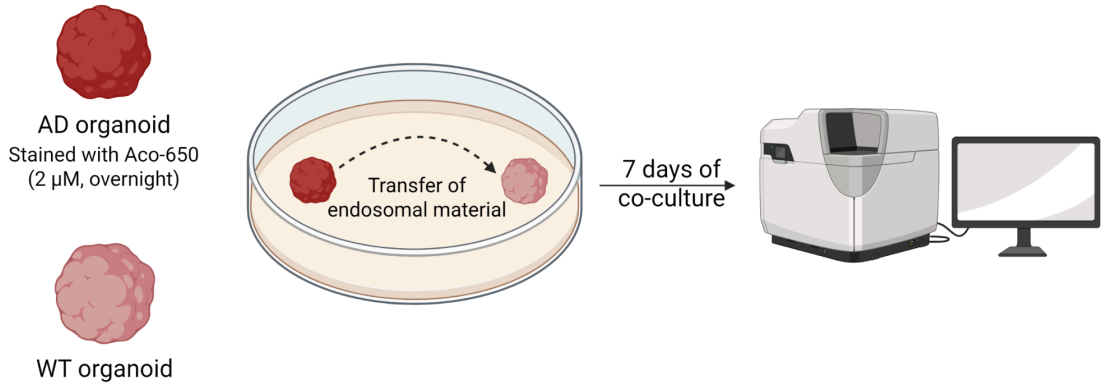


Figure 1: AD organoid was stained with Aco-650 overnight before being co-cultured with an unstained WT organoid. The organoids were imaged after 7 days of co-culture.

Materials and Methods

Full methodology can be found in the preprint by Balistreri *et al.*[1]. Key methodology involving Aco-Dyes are provided in brief.

Cell and Organoid Culture

Isogenic human induced pluripotent stem cell (hiPSC) lines (both WT and PSEN1^{M146V}/WT familial AD mutant) derived from a male donor were obtained from the Tessier-Lavigne laboratory and New York Stem Cell Foundation (Paquet et al. 2016).

hiPSCs were dissociated with Accutase, aggregated in Aggrewell plates with mTeSR Plus + CEPT (Chroman I, Emricasan, Polyamine supplement, and Trans-ISRIB) for 24 hours, before being transferred to ultra-low attachment plates on an orbital shaker (60 rpm). The resultant embryoid bodies were cultured in Essential 6/hESC medium with WNT/Dual SMAD inhibition for 6 days, followed by in Neural Media (NM) supplemented with EGF2/FGF2. Between days 25 and 43, the growth factors were replaced with neurotrophic factors BDNF and NT-3. The differentiated organoids were then maintained long term in NM (Neurobasal™-A Medium, B-27™ Supplement, minus vitamin A, 1% GlutaMAX™ Supplement).

Confocal Imaging of Organoids

AD organoids were incubated overnight in NM supplemented with 2 µM Aco-650 (Acoerela). After overnight staining, the organoids were rinsed three times with PBS. The Aco-650-labelled AD organoids were co-cultured with a WT organoid in the same well for 7 days, with fresh media replaced every other day. For experiments involving the suppression of endosomal material transfer, 500 nM GW4869 was added. On the 7th day, all organoids were removed from co-culture, briefly stained with Hoechst nuclear stain by incubating the organoids in NM

supplemented with the Hoechst nuclear stain (1:4000 dil., 62249) for 10 mins. After three rinses with PBS, the organoids were moved to BrainPhys™ Imaging Optimized Neuronal Medium (05796) and placed in an optical bottom 96 well plate (07000629). The organoids were imaged using a Zeiss Celldiscoverer 7 microscope with the specimen chamber set to 37 °C and 5% CO_2.

Results

The organoids were first stained with 2 µM Aco-650 overnight, followed by three PBS washes and Hoechst staining. Confocal imaging was then performed to validate the staining (Figure 2).

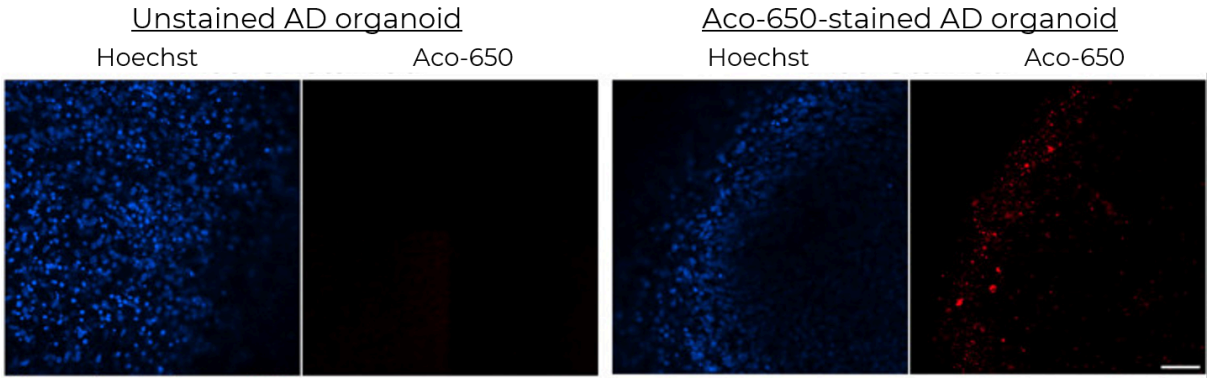


Figure 2: Confocal images of unstained and Aco-650-labelled AD cerebrocortical organoids. Scale bar: 50 µm

After validating that the organoids could be stained with Aco-650, the stained AD organoids were co-cultured with WT organoids for 7 days. As a control (to detect any autofluorescence of the organoids), the WT organoids were also co-cultured with another unstained WT organoid. During the 7 days, the media was replaced every other day. The WT organoids were imaged to determine the Aco-650 signals (Figure 3).

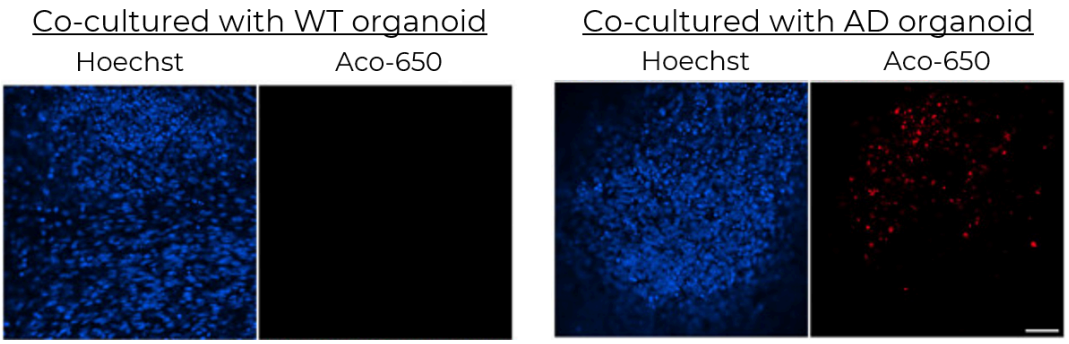


Figure 3: Live-cell imaging of WT cerebrocortical organoid after being co-cultured with another WT organoid or an Aco-650-labelled AD organoid for one week. Scale bar: 50 µm

To further validate that the presence of Aco-650 fluorescence signals is attributed to the transfer to endosomal materials, an inhibition study was performed. GW4869 was used to reduce

exosome generation and release by inhibiting ceramide-mediated multivesicular body budding. In the presence of 500 nM GW4869, there is a significant reduction in fluorescence signals observed in the WT organoid (Figure 4). This implies that the transfer of endosomal materials partially contributed to the presence of Aco-650 fluorescence signals observed in the WT organoid after 7 days of co-culture.

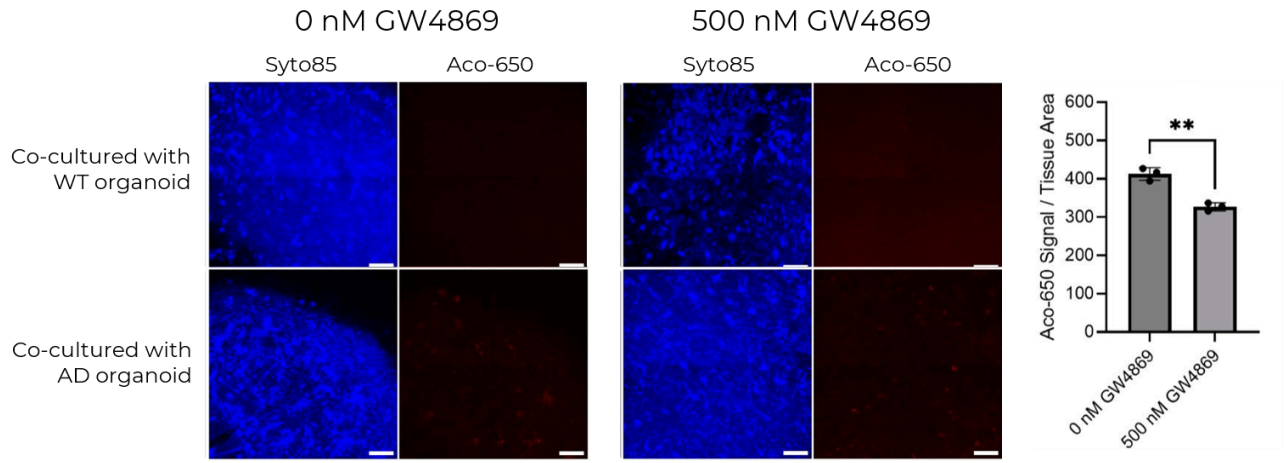


Figure 4: Live-cell imaging of WT cerebrocortical organoid after being co-cultured with another WT organoid or an Aco-650-labelled AD organoid for one week in the presence or absence of 500 nM GW4869. Scale bar: 50 µm. Aco-650 signal per tissue area was quantified (n = 3).

Conclusion

Aco-650 demonstrated successful labelling of organoids. In this study by Balistreri *et al.*[1], the presence of Aco-650 signals in the unstained organoid co-cultured with Aco-650-labelled organoid suggests organoid-organoid interactions and communication. The inhibition study using GW4869 validates that the transfer of endosomal materials partially contributes to the detection of Aco-650 signals in the unstained organoids. Overall, this workflow provides an approach to investigate the mechanism of disease progression through communication and transfer of endosomal materials.

In the broader study, Balistreri et al. characterized small EVs collected non-invasively from the conditioned media of AD and WT cerebrocortical organoids[1]. AD organoids released roughly twice as many EVs as WT organoids. Their EVs had a wider size distribution, an altered tetraspanin profile, and multilamellar and fibril-associated morphologies by cryo-TEM. Proteomic profiling of AD EVs showed changes in autophagy and membrane-trafficking proteins, consistent with a defect in secretory autophagy. EVs from both genotypes bound the amyloid-detecting dyes ThT and AmyTracker 680. In the Aco-650 co-culture experiments, WT organoids exposed to AD organoids also showed more intratissue Aβ puncta than WT organoids co-cultured with WT organoids, and GW4869 reduced this increase. Together, these results suggest that amyloid-associated material can move between tissues through EVs.

Guidelines for Using Aco-Dyes

1. Aco-Dyes vary in intensity and staining efficiency. It is recommended to optimise the method for each dye, cell type, organoid size and study condition.
2. Conduct a titration study to determine the optimal concentration and duration to label the cells or organoids. Suggested concentration range: 1 to 20 µM. Suggested duration range: 24 to 48 hours.

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References

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