#196.07 - TOWARDS NEW RELEVANT ALZHEIMER'S DISEASE MODELS FOR TARGET VALIDATION AND DRUG TESTING

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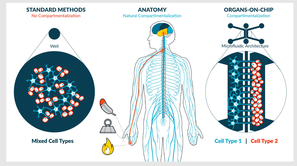
BACKGROUND



Over the past decade, **no molecules tested in clinical trials** to **slow or cure neurodegenerative diseases have been brought to market**. We present here an **essential first step** towards the development of innovative **organs-on-chip (OoC) models of Alzheimer's disease**, in order to elucidate the underlying mechanisms of the disease and to search for new effective therapies. This innovative high-throughput brain-on-chip platform uses:

- **Compartmentalized co-culture** of glutamatergic and GABAergic neurons derived from human induced pluripotent stem cells (hiPSCs) **with the fluidic isolation of NETRI's DuaLink MEA device**
- The addition of ETAP-Lab's **oligomeric forms of amyloid beta$_{1-42}$ (AβO)** in one of the three channels (work in progress).

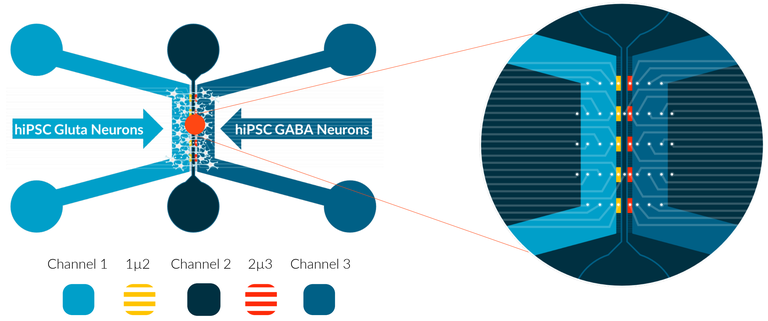
EXPERIMENTAL DESIGN

COMPARTMENTALIZED MEA-CAPABLE OoC DEVICES

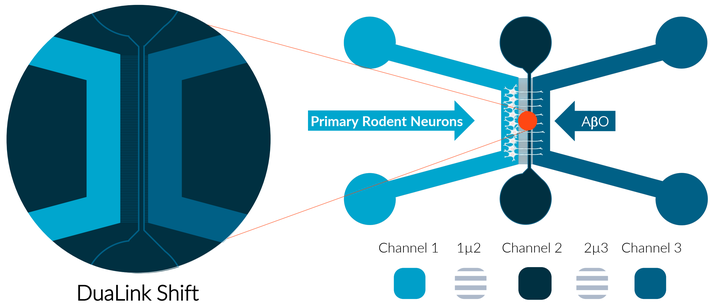
Co-culture of hiPSCs neurons:

- hiPSC-derived glutamatergic neurons (BX-0300) in Channel 1
- hiPSC-derived GABAergic neurons (BX-0400) in Channel 3
- Oligomers and/or compounds applied in Channels 1, 2, or 3
- Response recorded in all channels and microchannels

BrainXell
AXION BIOSYSTEMS



AßO-INDUCED NEURODEGENERATION



Culture of primary rodent neurons:

- Primary cortical neurons in Channel 1
- At Day 21, AβO challenge for 4 days in Channel 3
- Response analysed in Channel 1 and Channel 3: staining β-tubulin/DAPI and imaging with Operetta CLS High Content Analysis System from REVVITY

ETAP-Lab unique know-how:

- Production of stable and soluble oligomers from their human native proteins Aβ$_{1-42}$
- High reproducible neurotoxicity reversed by reference compounds

RESULTS

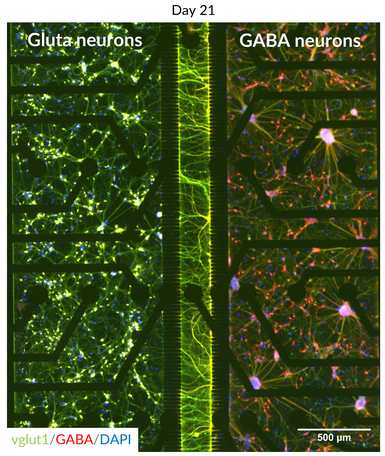
RELEVANT HEALTHY ALZHEIMER'S MODEL

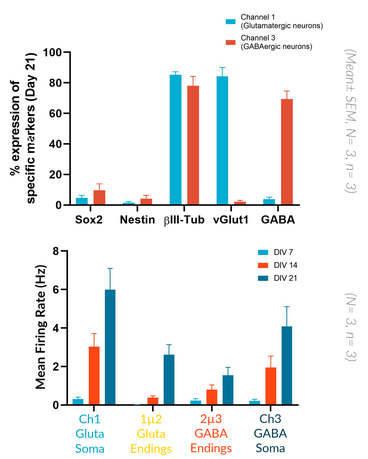
Expression of markers by semi-automated quantification using NETRI's proprietary software:

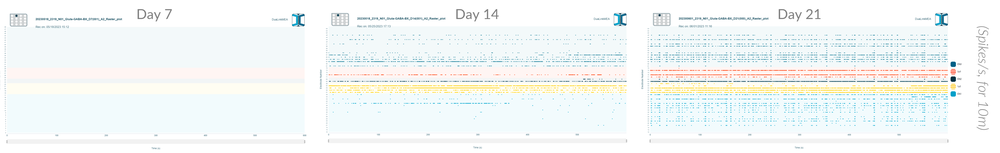
- More than 70% of phenotypic markers (Day 21)
- Less than 10% of pluripotency markers (Day 21)

Increase of firing rate in the neuronal networks-on-chip:

- No cell damages after three recordings (up to Day 21)
- Recording of electrophysiological activity from day 14







RELEVANT INJURY ALZHEIMER'S MODEL

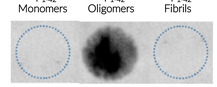
Oligomers for more translational value:

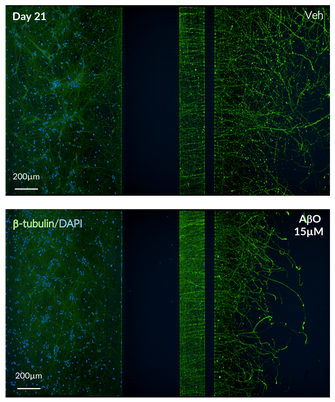
- Oligomer-specific antibody (A11) recognized only AβO (Dot-blot assay)
- Fluorescence intensity increased only upon binding AβO not monomers or fibrils (ANS assay)
- SV-AUC study showed only 8% of monomers in mixture oligomers species, the rest is dimers (60%), trimers (20%) and higher molecular weight species (12%)

Localized axonal degeneration:

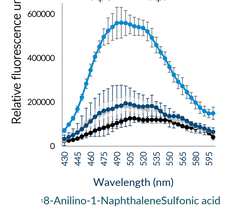
- Axonal challenging with AβO induced axonal degeneration in dose-dependent manner
- Soma showed no sign of neurodegeneration after local AβO deposits

A11 dot-blot Oligomer antibody



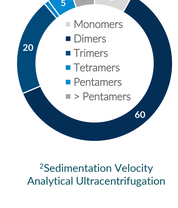


ANS[1] fluorescence emission spectra of AβM, AβO & AβF



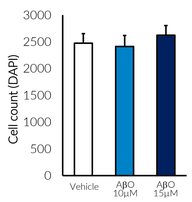
[1]8-Anilino-1-NaphthaleneSulfonic acid

SV-AUC[2] Oligomers mix characterisation

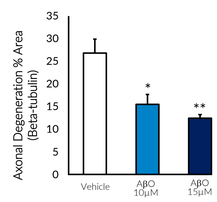


[2]Sedimentation Velocity Analytical Ultracentrifugation

Cell quantification in Channel 1 post local AβO addition



Axonal quantification in Channel 3 post-AβO challenge

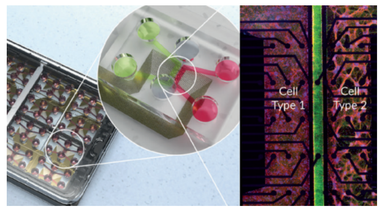


All values are mean ± SEM. Statistical significance level: * P<0.05 and ** P<0.01 vs Vehicle (One-Way ANOVA followed by Scheffé post-hoc), n=4

CONCLUSION & PERSPECTIVES

By combining **NETRI's engineering, biological, and digital expertise** with **ETAP-Lab's expertise** in the **modeling of neurological diseases** on rodent cells and hiPSCs, and in the **development and manufacture of neurotoxic oligomers**, we have set up a **model of Alzheimer's disease-on-chip**.

- Fully differentiation and maturation of human neurons on-chip
- Standard Operating Protocol of human glutamatergic and GABAergic neurons co-culture
- Protocol to induce AβO injury on-chip with rodent cells and hiPSC-derived neurons (in progress)



After developing a model of Alzheimer's disease-on-chip, we will focus on the **injury model on hiPSCs** and the **extraction of digital signatures**, using our **UpLink™ utility software**, to finally **add reference compounds and compounds of interest**.

Our **Brain-on-Chip platform** will offer pharmaceutical companies and researchers a **new model for preclinical studies, enabling them to reproduce complex neuropathological phenomena finely**.

BIO-DIAMOND

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