Amyloid-beta Induces Toxicity and Cell Death in Human iPSC-derived Neurons: Alzheimer Disease *In Vitro* Model

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1 INTRODUCTION

Alzheimer's is a genetic chronic neurodegenerative disease that typically begins around the age of 60 and progressively impairs cognition and language. A key common hallmark is the accumulation of plaques containing β-amyloid that leads to synaptic failure and, eventually, neuronal death.
In recent years, reproducing and studying the mechanisms behind Alzheimer's disease's (AD) pathology and β-amyloid plaques-dependent degeneration have been facilitated by the advent of induced pluripotent stem cells (iPSCs).

The goal of the current study is to develop a robust AD *in vitro* model, based on the treatment of iPSC-derived glutamatergic neurons with commercially available β-amyloid aggregates.

2 METHODS

Bit.bio ioGlutamatergic neurons were derived from human induced pluripotent stem cells (iPSCs), precision reprogrammed using opti-ox™ technology. Pre-differentiated neurons were thawed and cultured for 9 days to allow full maturation. Next, cells were exposed to 5, 10, 20 or 40 µM β-amyloid aggregates for 72 hours and cell toxicity was evaluated by:
1) Immunohistochemistry: on day 12 cells were fixed, and immunocytochemistry was performed for DAPI and βIII-tubulin. High content imaging was done using the Yokogawa CV8000 and PE Columbus 2.9.1 was used to create an algorithm for quantification, focusing on nuclei size and signal intensity (DAPI) and neuritic structures.
2) Meso Scale Discovery (MSD): assay performed according to the supplier manual, measuring Neurofilament Light Chain (Nf-L) release in the supernatants

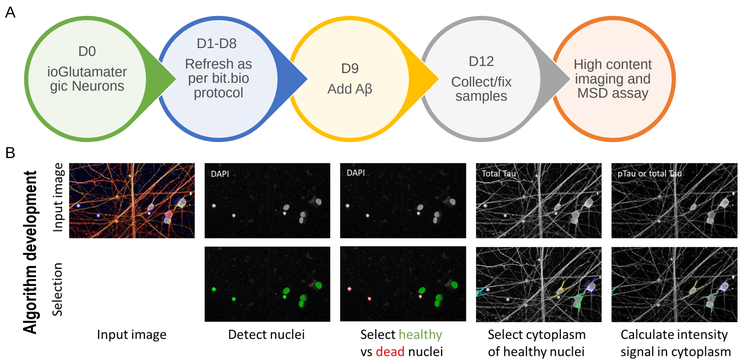

Figure 1. Schematic representation of experimental setup of assays performed to evaluate the impact of the exposure of ioGlutamatergic neurons to β-amyloid aggregates. *(A) Workflow and experimental timelines (B) High content imaging and analysis was performed using the Yokogawa CV8000 and an algorithm that was developed in-house using PE Columbus 2.9.1 for quantification.*

3 RESULTS

Toxicity assessment by High Content Analysis

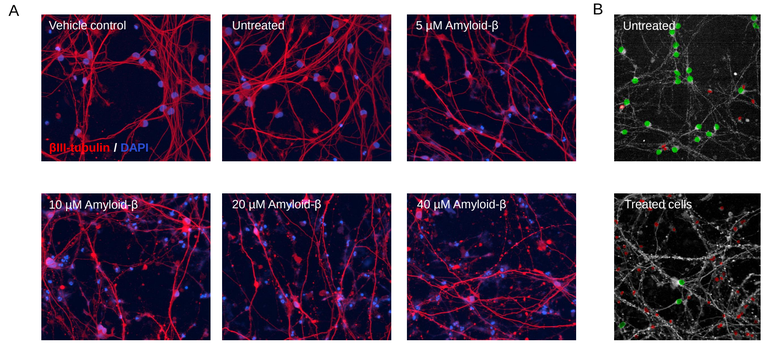

Figure 2. Exposure of ioGlutamatergic neurons to β-amyloid aggregates impaired neurite structure and the number of healthy nuclei. *(A) Representative images of ioGlutamatergic neurons exposed for 72 hours to different concentrations (0-40 µM) of β-amyloid aggregates and stained for βIII-tubulin (red) and DAPI (blue). (B) Examples of nuclei segmentation in ioGlutamatergic neurons not treated or treated with β-amyloid aggregates. Green dots: healthy nuclei; red dots: unhealthy, condensed nuclei. High content imaging was performed using the Yokogawa CV8000.*

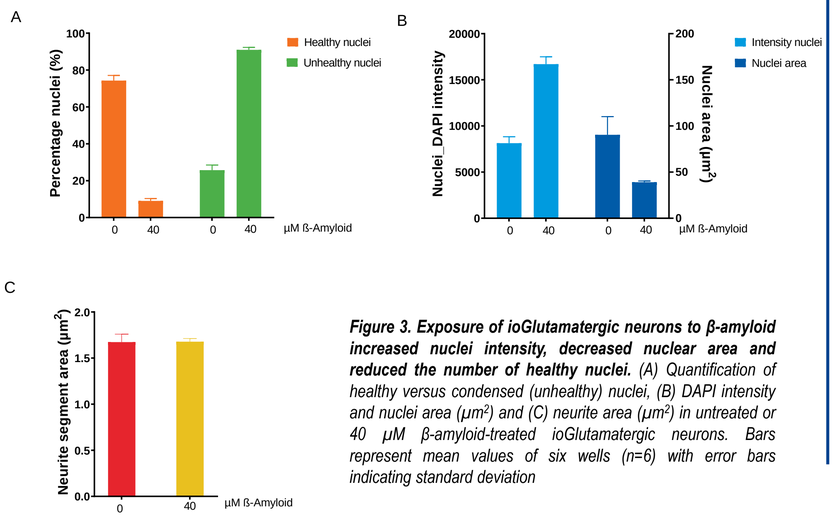

Figure 3. Exposure of ioGlutamatergic neurons to β-amyloid increased nuclei intensity, decreased nuclear area and reduced the number of healthy nuclei. *(A) Quantification of healthy versus condensed (unhealthy) nuclei, (B) DAPI intensity and nuclei area (µm²) and (C) neurite area (µm²) in untreated or 40 µM β-amyloid-treated ioGlutamatergic neurons. Bars represent mean values of six wells (n=6) with error bars indicating standard deviation*

Toxicity assessment by MSD analysis of Neurofilament Light Chain (Nf-L)

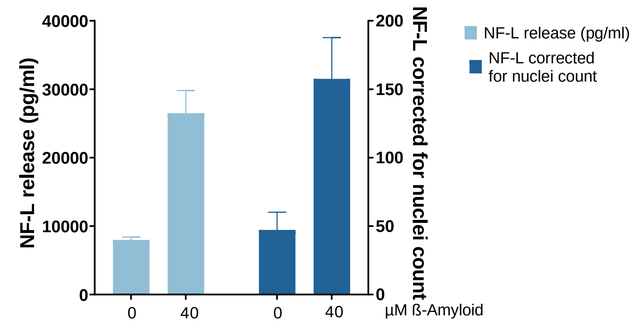

Figure 4. Exposure of ioGlutamatergic neurons to β-amyloid aggregates induced Nf-L release. *Quantification of total Nf-L release by MSD in untreated or 40 µM β-amyloid-treated ioGlutamatergic neurons. Light blue bars depict Nf-L release as measured by MSD analysis in supernatants. Dark blue bars depict Nf-L release normalized to cell number (nuclei count quantified by high content analysis). Bars represent mean values of six wells (n=6) with error bars indicating standard deviation.*

4 CONCLUSION

In conclusion, Charles River has successfully developed a robust Alzehimer's Disease *in vitro* model, in which treatment of iPSC-derived glutamatergic neurons with commercially available β-amyloid aggregates led to a quantifiable reduction of neuronal viability in line with patient pathology.

Compared to the vehicle control and untreated cells, exposure of neurons to β-amyloid for 72 hours induced toxicity, as shown by the destruction of neurite structures (stained for β-III tubulin) and the reduction of DAPI-positive healthy nuclei. These effects were observed in a β-amyloid concentration-dependent manner and confirmed by quantification of 40 µM β-amyloid-treated samples using high content analysis. Neurodegeneration was further confirmed by a higher release of Neurofilament Light chain (NfL) in 40 µM β-amyloid aggregate-treated neurons.

Taken together, these preliminary results support the validity and strength of this model and open the path for future disease-relevant applications, including compound screening, with the goal of establishing effective treatments for AD.