Development of a Neuronal Viability Assay Using SH-SY5Y Cells

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Abstract

Alzheimer's disease affects more than 35 million people internationally and accounts for 50-70% of all dementia cases[1,2]. This is a compounding problem as the general population is aging and living longer. There are many potential causes of dementia, but one of the most striking is neuronal cell death. Viability assays are an important scientific tool that allows one to compare the toxicity of harmful substances of interest by a standard of cell death using precise conditions and measurement techniques. Here, a viability assay was created using the human neuroblastoma cell line, SH-SH5Y, and increasing concentrations of hydrogen peroxide (H_2O_2) were used to elicit cell stress and cytotoxicity. Neurotoxicity was measured using flow cytometry in combination with a proprietary live/dead dye. Previously, phosphorylated tau protein oligomers have been shown to be neurotoxic, and possibly be one of the steps that is responsible for eliciting dementia and potentially Alzheimer's[3]. Good *et al.* has shown that Aβ oligomers are neurotoxic to cultured SH-SY5Ys[4]. This work will provide assistance for future experiments in which a pure tau sample can be obtained and assayed for cytotoxicity.

Tau Aggregation

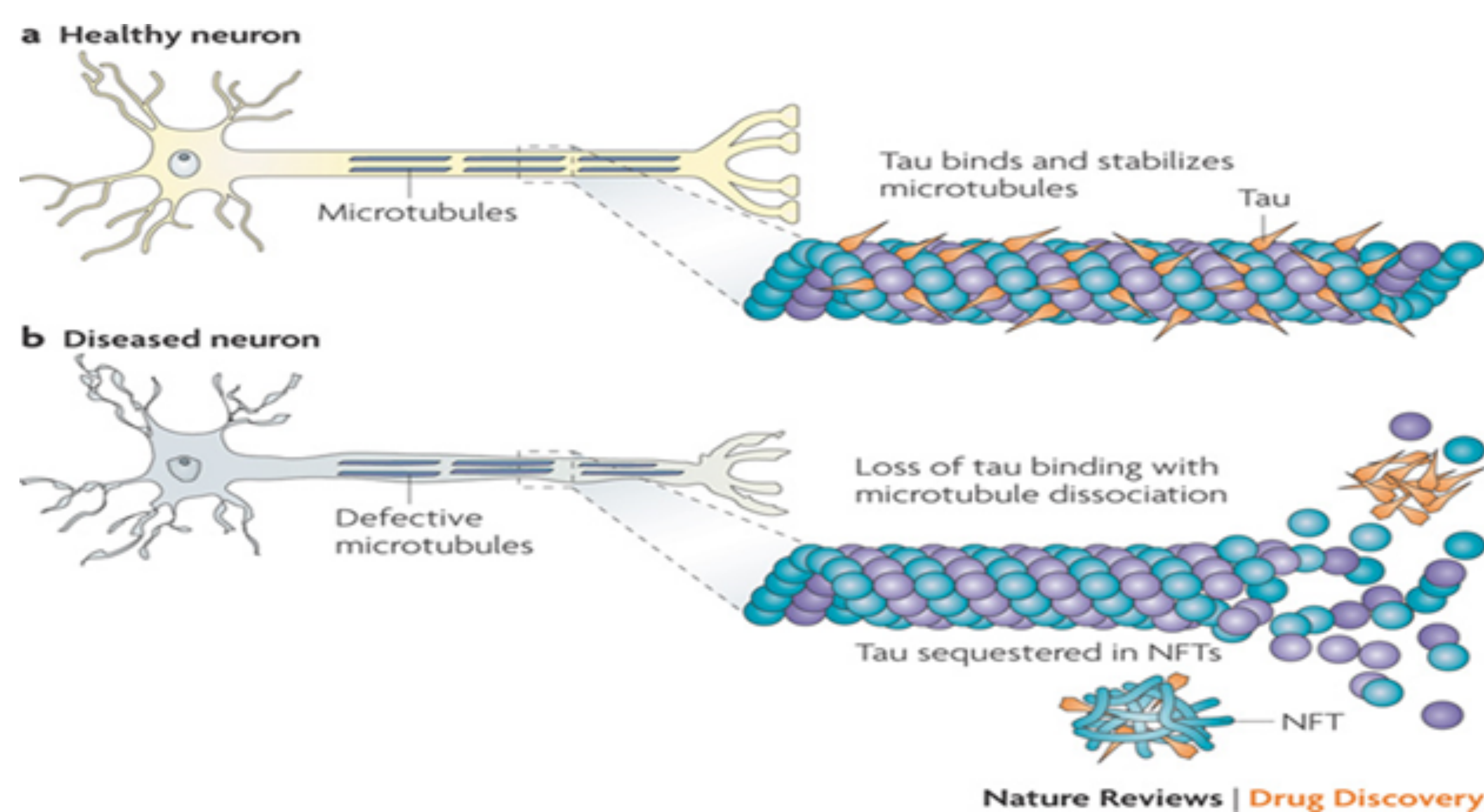

Figure 2[5]. The effect of hyperphosphorylated tau on neurons. Tau protein functions to stabilize microtubules normally, but when tau becomes hyperphosphorylated, it tends to fall off and form oligomers. This destabilizes microtubules, inhibits cellular transport, and decreases neuronal polarity. Furthermore, tau oligomers can be neurotoxic.

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Flow Cytometry

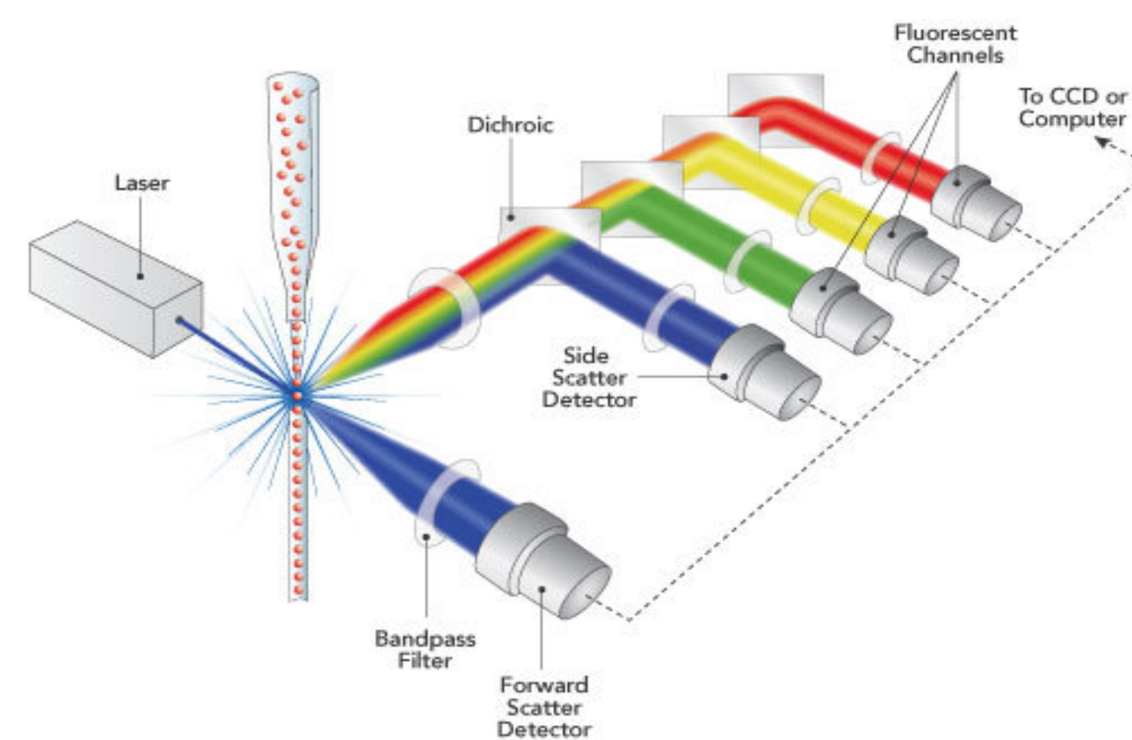

Figure 3[6]. The basics of flow cytometry. A suspension of cells is brought up by the machine and passed through a laser in a single-file line. The light is scattered as it passes through the cell, and based on the direction and intensity, the cytometer can determine relative size, complexity, and florescence if available. A major advantage to flow cytometry is obtaining quantifiable data.

SH-SY5Y Cells

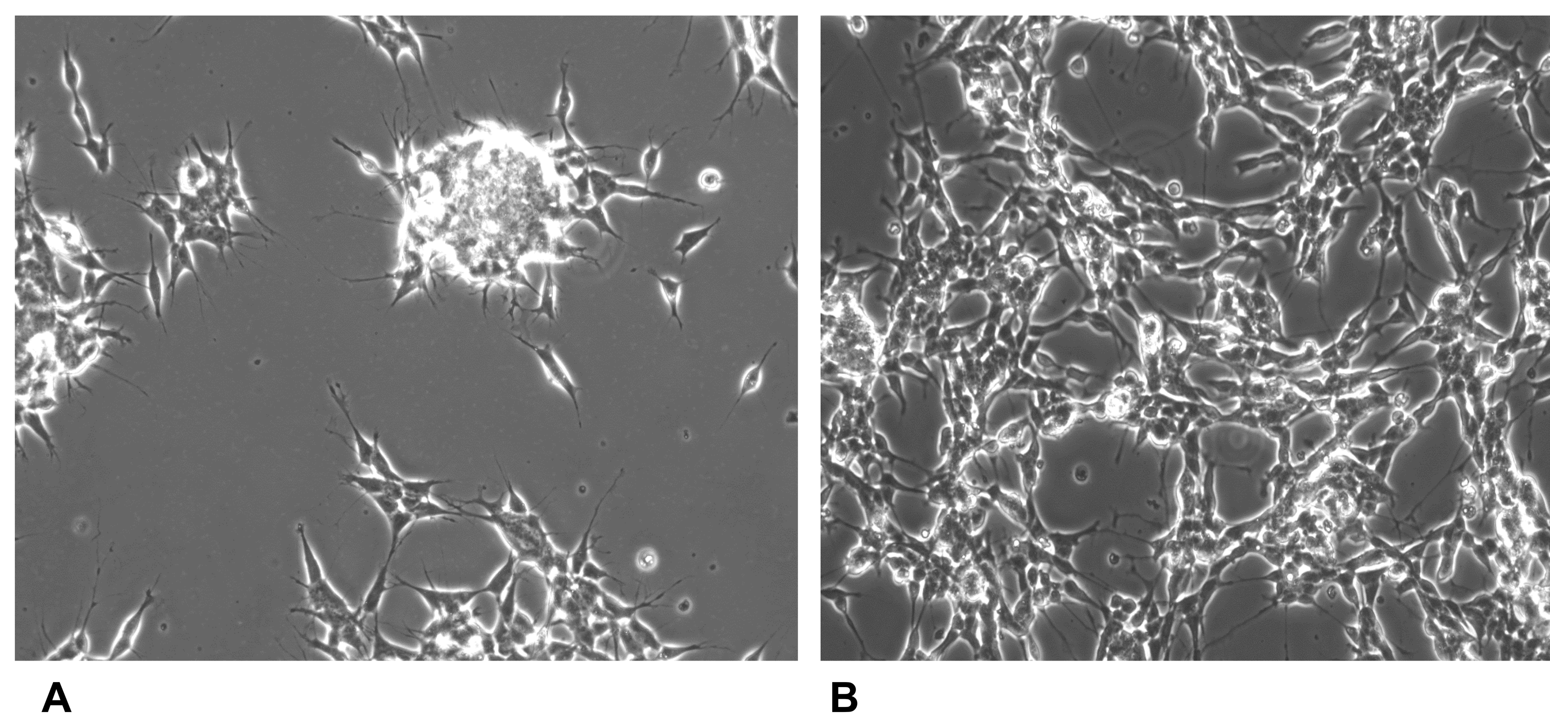

Figure 1. (A) Undifferentiated SH-SY5Y cells at 10x. Cells are less specialized and less neuronal-like than fully differentiated cells. They will also form larger, mound-like structures. If left alone to grow in appropriate media, they will become too confluent and die. (B) Differentiated SH-SY5Y cells at 10x. The cells were differentiated with 10 μM retinoic acid (a form of Vitamin A) and left to grow for 1-5 days before experimentation. Differentiated SH-SY5Y cells form more synapses and tend to be longer, more pyramidal, more neuronal-like, and have more complex extensions. They also express a higher concentration of neuronal markers, such as choline acetyl transferase (ChAT) and vesicular monoamine transporter (VMAT)[7].

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Viability Assay

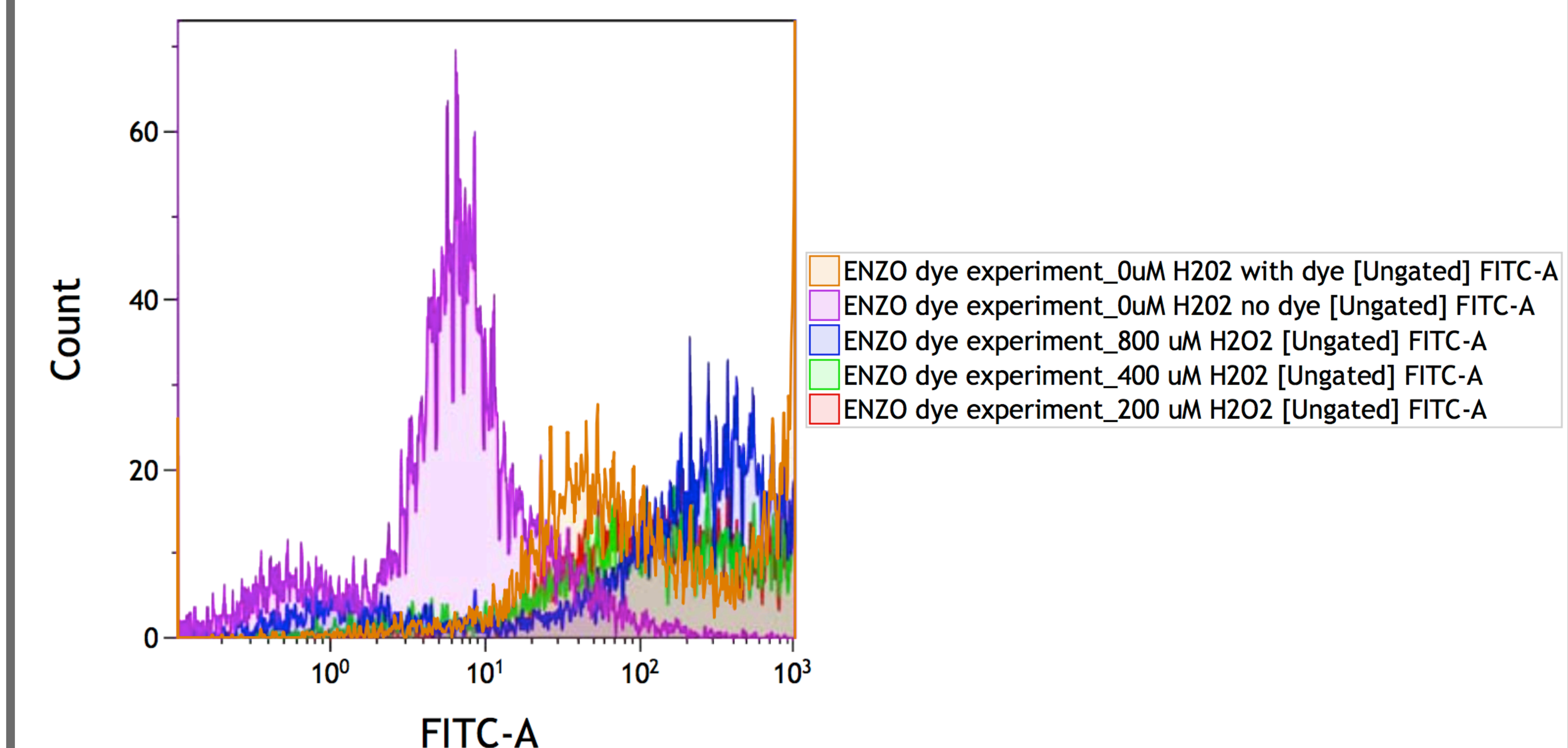

Figure 4. Overlaid histograms representing the flow cytometry data from a viability assay using a live/dead assay dye obtained from Enzo® Life Sciences. The Enzo dye contains a green fluorescent dye that is excluded by live cells and included by dead cells. Viability assays were done by first seeding a 6-well plate with undifferentiated SH-SY5Y cells. Once these cells adhered to the wells, they were differentiated with 10μM retinoic acid and allowed to grow for another 2 days, changing media whenever necessary. After differentiation, stress media consisting of 0, 100, 200, 400, and 800 μM H_2O_2 were applied to the different wells and incubated for 2 hours. After incubation, the Enzo dye was applied in phosphate buffered saline (PBS), and then incubated for 30 minutes. After this, the wells were scraped and resuspended in PBS before being analyzed in the flow cytometer.

Future Work

- **Obtain pure tau samples and develop a method to promote oligomerization**
- **Perform a viability assay on differentiated SH-SY5Y cells similar to the one described in this experiment**
- **Compare flow cytometry data from stress viabilities to oligomerized tau viabilities**
- **Correlate tau and stress concentrations to cell death in order to attain a relative neurotoxicity of tau oligomers**

References

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