IDENTIFICATION OF HSV-1-INDUCED PLAQUE-LIKE FEATURE FORMATION ON A 3D NEURAL STEM CELL CULTURE MODEL IN ALZHEIMER'S DISEASE

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BACKGROUND

Alzheimer's Disease is a neurodegenerative disorder that afflicts more than 50 millions individuals worldwide, demonstrating severe cognitive decline, memory loss, confusion and deterioration of mental function.[1]

Important hallmarks of AD are the formation of senile amyloid plaques (misfolded proteins) and neurofibrillary tangles (NFTs).[2]

Two-photon excited fluorescence (TPEF) is a non-destructive, non-invasive imaging method which will be used herein to analyze metabolic changes associated with AD development and plaque formation.[3]

Previous work conducted by the Kaplan Lab has established a 3D brain-like model that exhibits AD-like physiology when infected with low levels of herpes simplex virus type I (HSV-1).[4]

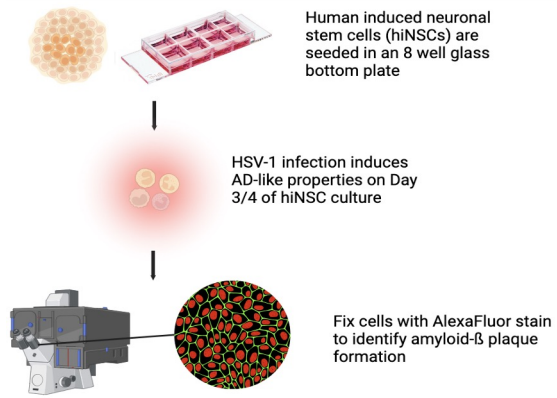
PURPOSE

This preliminary study aims to establish an efficient image acquisition protocol using 2D human induced neural stem cell (hiNSC) monolayer cultures and applied to the 3D brain-like tissue model. Optimizing this procedure will enable us to conduct future analysis with the 3D brain-like model of AD in a non-destructive and efficient manner, allowing us to better characterize the dynamic metabolic changes that occur through disease development.

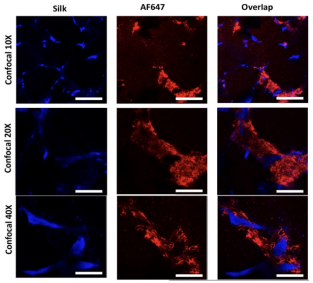
METHODS

Images were acquired using a Leica TCS SP8 confocal microscope. Regions of interest were first identified by performing a preliminary scan of each specimen using the 10x air objective. The microscope was adjusted to locate the best imaging depth for locating plaque-like formations (PLFs). After identifying the PLF, further adjustments were made to center the ROI in the field of view. The X, Y, and Z positions were recorded before the microscope was switched to a 20x air objective. Under 20x magnification, the prerecorded coordinates were used to locate the same ROI before new coordinates were registered. Finally, the microscope was switched to a 40x water-immersion objective.

A 3D brain-like tissue model of AD was established as previously described.[4] *Bombyx mori*-derived silk solution was utilized to create donut-shaped scaffolds. hiNSCs were allowed to attach overnight before 3D brain-like tissue constructs were cultured in neurobasal media and allowed to mature for 4 weeks.



RESULTS



Images acquired of hiNSC 3D cultures stained with AF647-conjugated Aβ+ PLFs antibody (red color). Silk was identified on DAPI channel (blue color). (*SB = 50 μm*).

CONCLUSION

The establishment of a PLF-identification protocol for 2D hiNSC cultures and the verification of the ability of this protocol to identify PLFs at 3D cultures will enable us to authenticate TPEF as a reliable and efficient imaging acquisition method that can correspondingly be utilized to analyze the 3D brain-like tissue model of AD.

Ultimately, optimizing this protocol serves as a novel method of identifying PLF development in a more robust model of the neurodegenerative disease, allowing us to further understand its metabolic development and pathophysiology.

FUTURE DIRECTION

Co-registration

- The corresponding data acquired of 3D hiNSC cultures stained with AF647-conjugated Aβ+ PLFs antibody will be geometrically aligned with their respective autofluorescent images from similar scaffold locations. This will enable us to identify their optical signatures to produce a more accurate portrayal of the PLFs.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my mentor, Maria Savvidou, and the Georgakoudi lab members for their support and guidance. I also greatly appreciate the Laidlaw Foundation for funding and supporting this work.

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