



Observation of α -synuclein propagation and pathological structure in human brain organoids by electron microscopy

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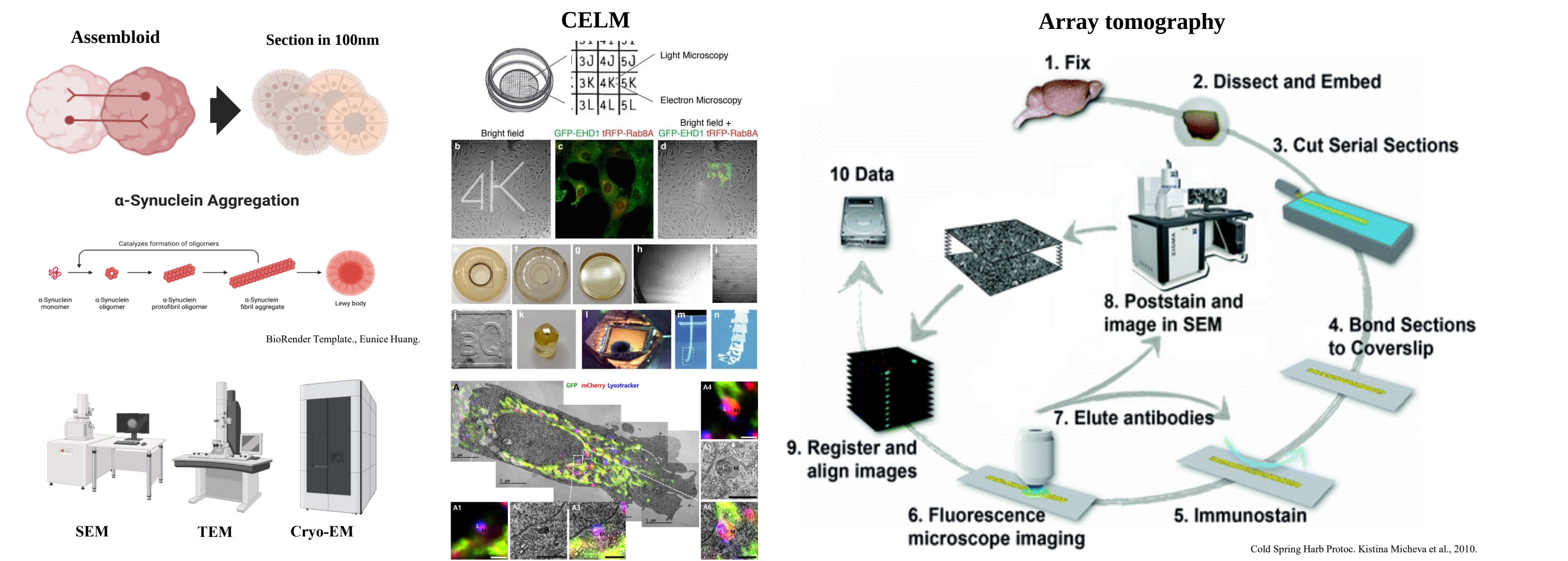
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Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the midbrain and the abnormal aggregation of α -synuclein (α -syn), forming Lewy bodies. These pathological aggregates disrupt cellular homeostasis, leading to neuronal damage and cell death, primarily affecting the dopaminergic pathways in the midbrain and striatum—regions closely linked to the motor symptoms of PD. Recent advances in three-dimensional brain organoid technology have facilitated the development of assembloid models that integrate the midbrain and striatum, allowing for a more patient-relevant platform to study the pathophysiology of PD. This model enables the simultaneous observation of dopaminergic neuron degeneration and α -syn aggregation, providing valuable insights into the disease's complex pathology. To investigate the structural and functional connectivity within this assembloid model, we employed large-area imaging CLEM (Correlative Light and Electron Microscopy). CLEM combines the wide-field capabilities of light microscopy with the high-resolution power of electron microscopy, enabling precise analysis of α -syn aggregation patterns, their intracellular localization, and their impact on organelle integrity and neural network connectivity. This study aims to explore the transport pathways and structural changes of α -syn aggregates and their interactions with cellular organelles in a midbrain-striatal assembloid model. We seek to provide a deeper understanding of the underlying mechanisms of PD pathology and identify potential molecular targets for therapeutic intervention.

Material and Method



- SEM and TEM** : Uses an electronic source and an electronic aperture; uses a series of electromagnetic and electrostatic lenses that control the shape and trajectory of the electron beam. However, The main difference between SEM and TEM is that SEM produces images by detecting reflected or knocked-out electrons, whereas TEM uses transmitted electrons (electrons that pass through the sample) to produce images. As a result, TEM provides important information about the internal structure of a sample, such as crystal structure, morphology, and stress state information, while SEM provides information about the surface and composition of a sample.
- Array tomography** : used to obtain high-resolution, 3D reconstructions of biological samples by imaging ultrathin serial sections. It allows for precise localization of multiple proteins or structures simultaneously while preserving the spatial context. This technique combines imaging modalities like fluorescence and electron microscopy for comprehensive molecular and structural analysis.
- Cryo-electron microscopy** : Uses an ion beam to create thin lamellae, which are then rapidly frozen at cryogenic temperatures below minus 180 degrees Celsius to preserve the original structure and rotated for more accurate three-dimensional imaging.
- CLEM** combines the two powerful techniques of light microscopy and electron microscopy to obtain detailed information about intracellular organelles from fluorescently labelled live or fixed cells.

Results

Structural analysis of α -syn in SH-SY5Y A53T cells using CLEM technique

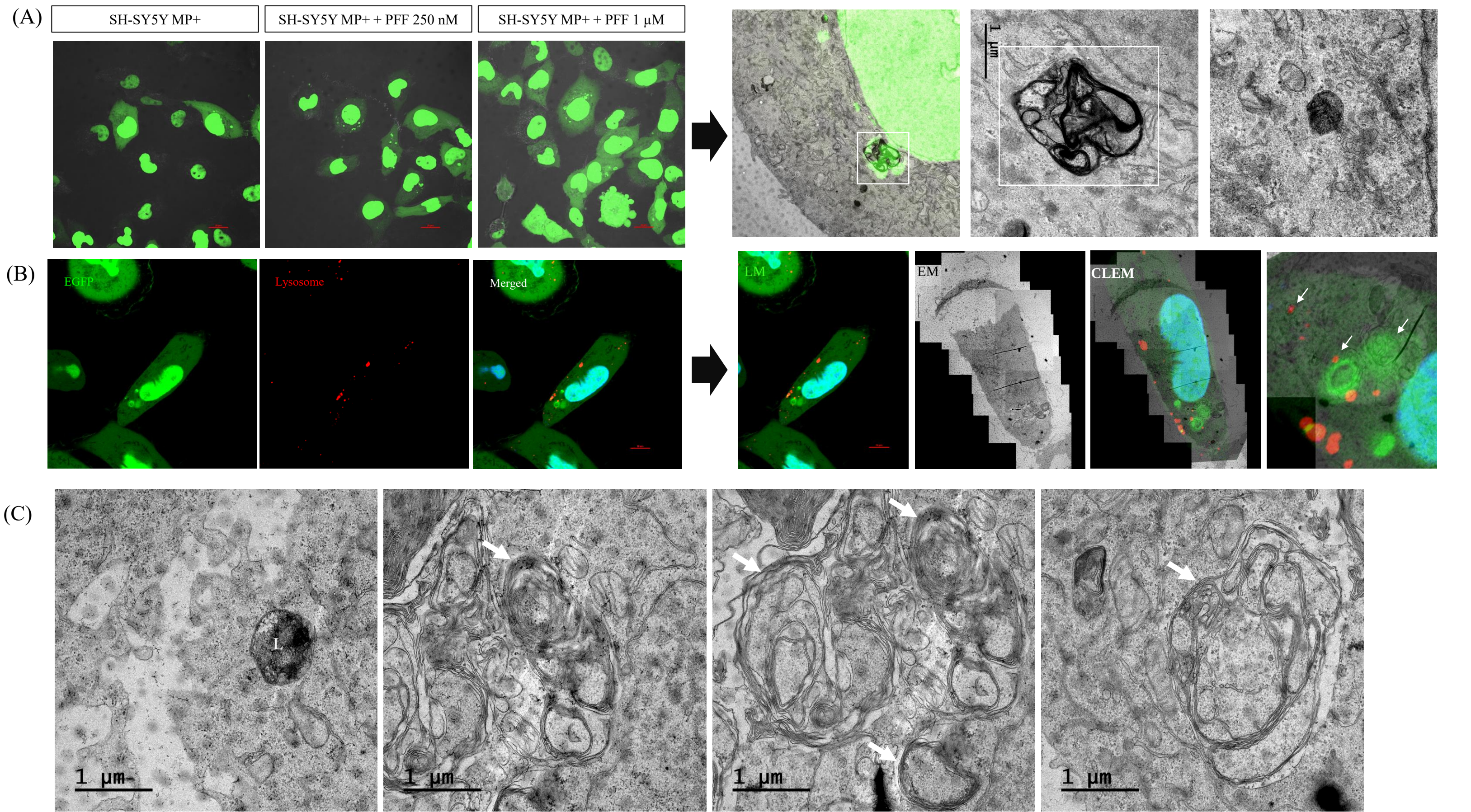


Figure 1. Analysis of α -syn aggregation in SH-SY5Y A53T-EGFP cells after PFF treatment using CLEM. (A-B) Representative CLEM images showing α -syn aggregates in SH-SY5Y cells treated with PFF for 24hr (green : α -syn, red : lysosome, blue : DAPI). (C) High magnification TEM image showing the ultrastructural feature of α -syn aggregates in the cells (L : lysosome, red arrow : α -syn aggregates). The cells and PFFs were provided by Professor Sangmyun Park's lab, Ajou University School of Medicine.

Large-area visualization of assembloid using SEM

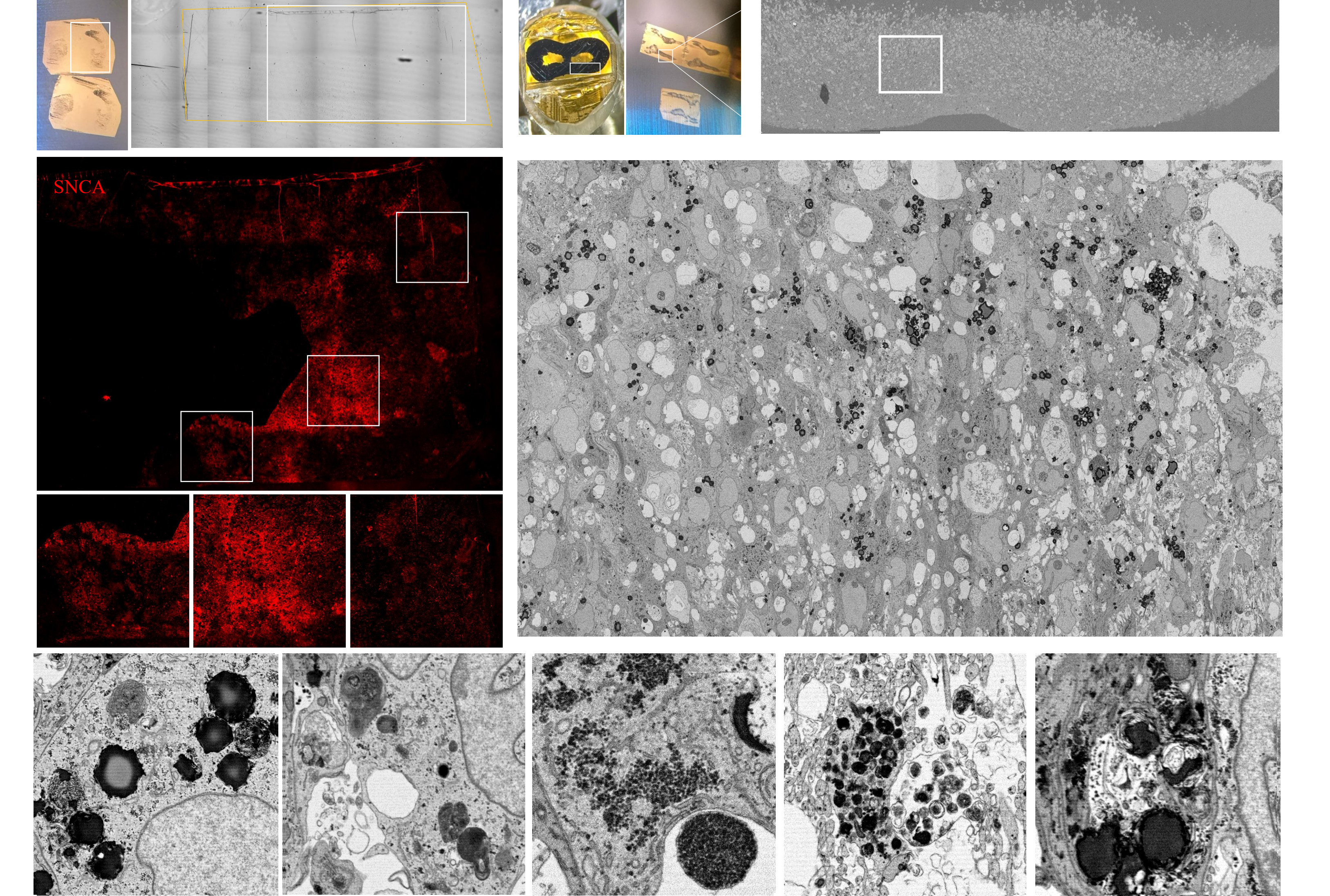


Figure 2. Large area image observation of α -syn accumulation in PFF-injected assembloid using SEM. Assembloid: Sample injected with PFF into the mid-brain. Original SEM image : 39003px X 24291px. 7Gb, imaging time : 6 hr

Results

Large image CLEM of injected with PFF in midbrain and SNCA overexpression assembloids

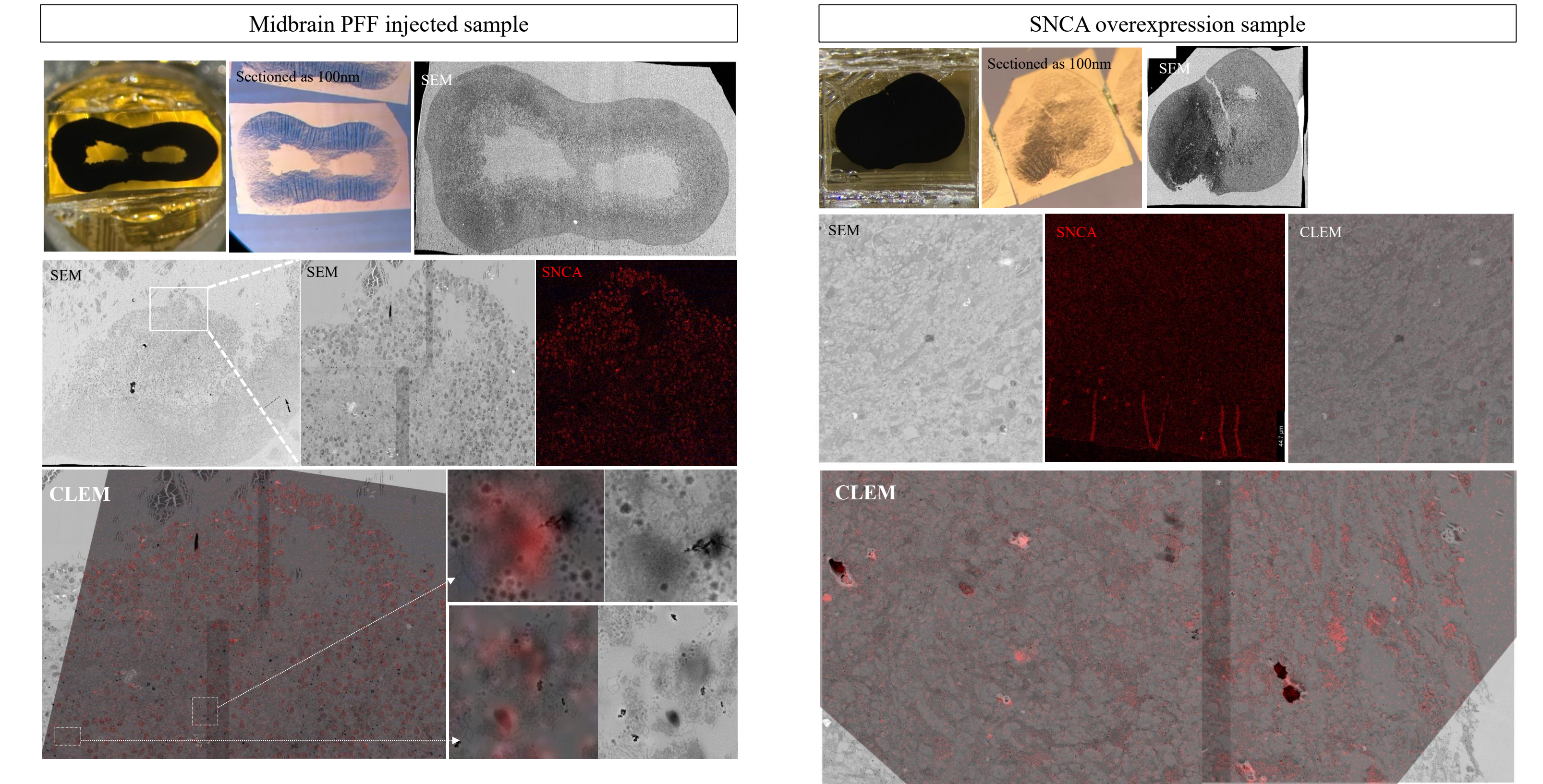


Figure 3. Comparison of α -syn aggregation in midbrain injected with PFF and SNCA OE assembloid using large-area CLEM.

Structural analysis of α -syn aggregates and Lewy body-like structures using TEM and SEM

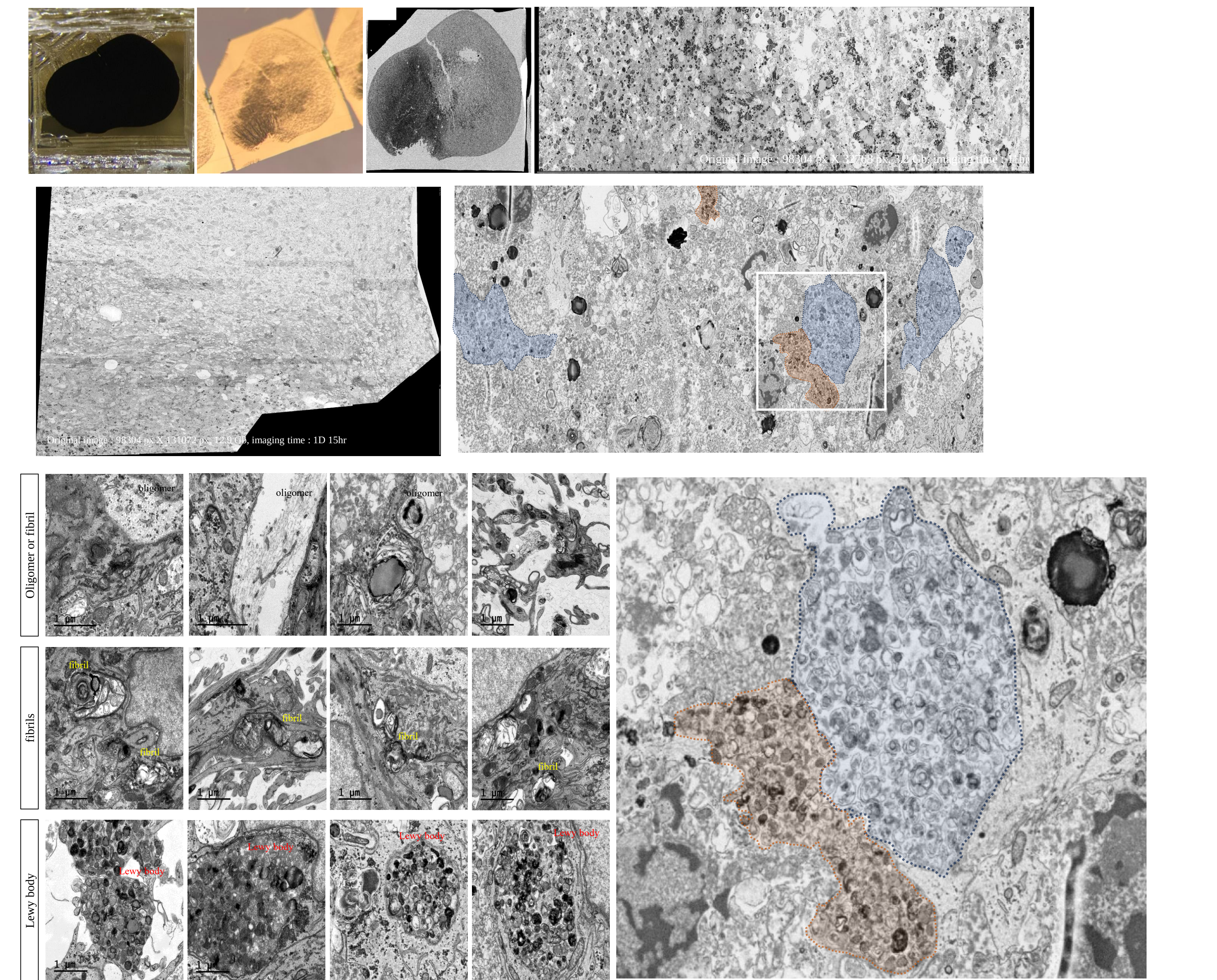


Figure 4. Comparison structural changes of α -syn aggregates and Lewy body-like structure in the SNCA OE assembloid using TEM and SEM.

Structural analysis of Lewy body-like structure using Cryo-tomography

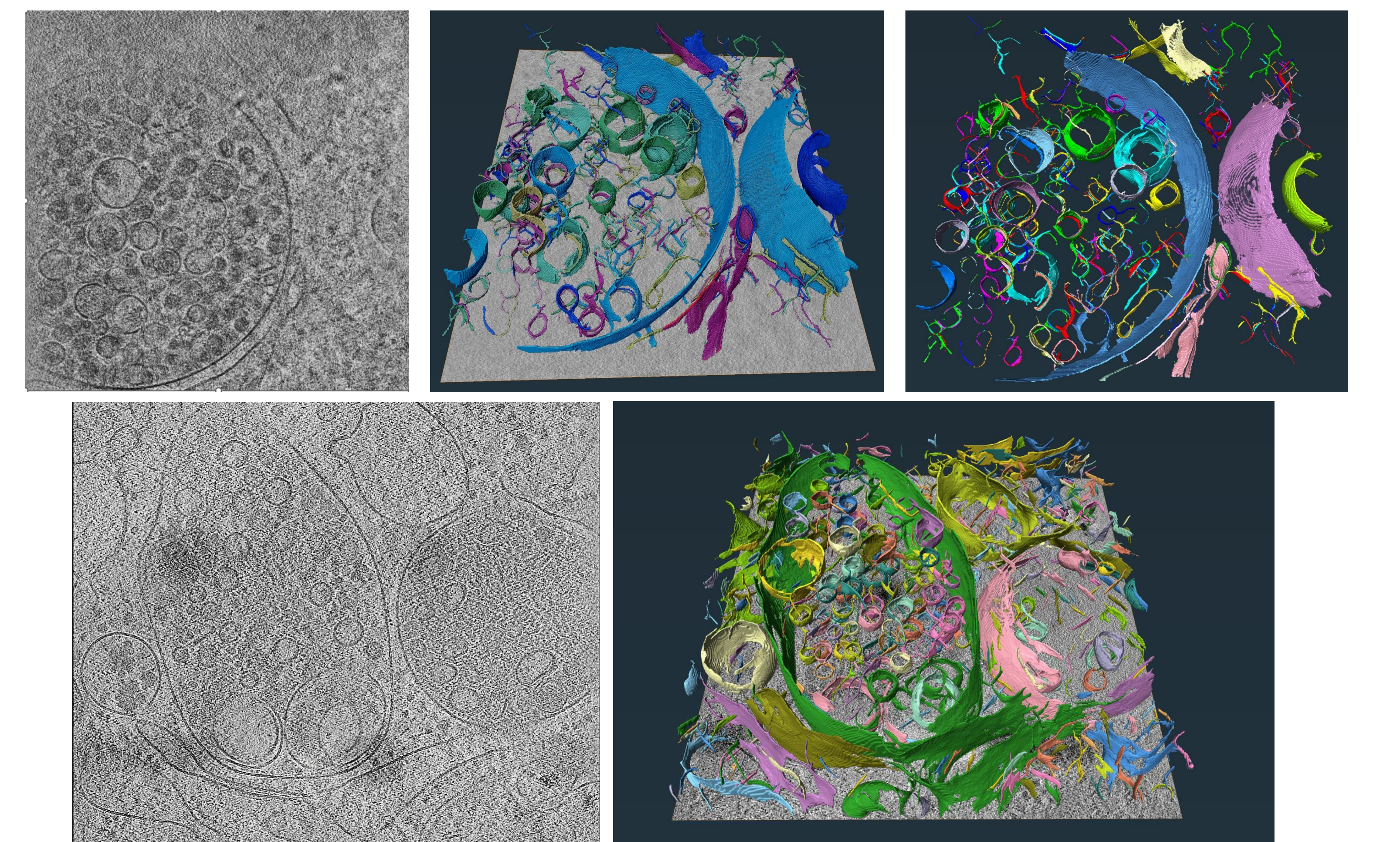


Figure 5. Characterization of Lewy body-like structure in A53T α -syn-EGFP SH-SY5Y cells using Cryo-electron tomography.

Summary

This study aimed to explore the transport pathways and structural changes of α -syn aggregates and their interactions with cellular organelles in a midbrain-striatal assembloid model. This provided a deeper understanding of the pathological mechanisms of dopaminergic neuronal degeneration in PD and suggested potential therapeutic targets. Future studies should focus on elucidating the exact pathways of α -syn propagation and exploring strategies to mitigate its toxic effects.

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