The Effects of Alzheimer's Disease Genotype on Tannic Acid-Coated Extracellular Vesicles for Targeted L-Carnitine Delivery

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss, cognitive decline, and neuronal dysfunction. Although amyloid-β accumulation, tau aggregation, and oxidative stress are strongly linked to AD progression, current treatments remain limited in their ability to stop or reverse the disease. One major challenge is the blood-brain barrier, which restricts delivery of therapeutic compounds to affected brain tissue. Extracellular vesicles (EVs) are promising drug carriers due to their biocompatibility, nanoscale size, and ability to cross biological barriers, but low loading efficiency and poor stability remain limitations. Tannic acid (TA) offers a potential coating strategy to improve vesicle function, while L-carnitine was selected as a model therapeutic cargo because of its role in mitochondrial metabolism and oxidative stress regulation. This study aims to develop and characterize TA-coated vesicles for improved therapeutic loading and delivery relevant to Alzheimer's disease.

Materials and Methods

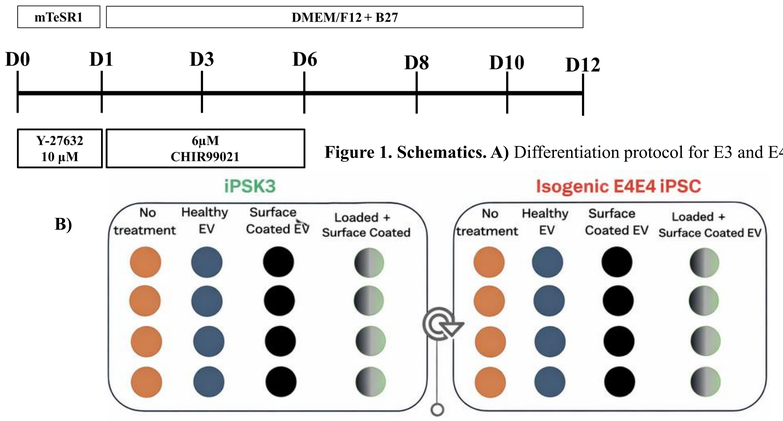


Figure 1. Schematics. A) Differentiation protocol for E3 and E4 iPSCs

To investigate the tannic acid surface-coated EV group, preliminary coating studies were first performed using synthetic liposomes as model vesicles. Liposomes provided a more controlled and reproducible system for optimizing the TA–Fe^{3+} surface coating before applying the method to biologically derived EVs, which are inherently more variable in size, composition, and membrane properties. This approach allowed coating formation and fluorescence-based surface characterization to be established first, creating the experimental foundation for the later evaluation of tannic acid-coated EV groups in the Alzheimer's disease model.

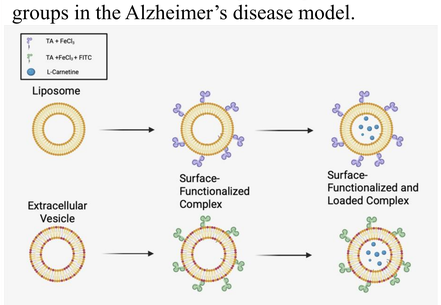


Figure 2 . Schematic of liposome and extracellular vesicle surface functionalization, illustrating tannic acid-based coating and loaded vesicle formation.

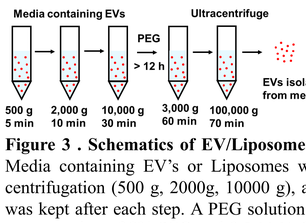
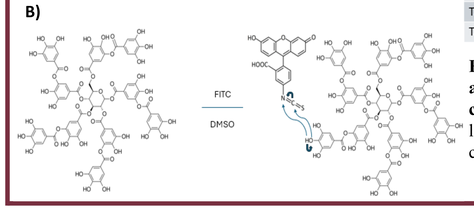


Figure 3 . Schematics of EV/Liposome isolation process. Media containing EV's or Liposomes went through serial centrifugation (500 g, 2000g, 10000 g), and the supernatant was kept after each step. A PEG solution was added at final concentration of 8% PEG and 0.5 M NaCl, and the solution was stored at 4°C overnight. On the second day, the pallet was kept after each centrifuge step.

B)



Total Liposomes: 2.9e10				
Trial	**% of Max**	**TA (mg)**	**$FeCl_3$ (mg, 25%)**	**FITC (mg)**
TA1	100	1.45	0.3625	0.1
TA2	75	1.0875	0.2719	0.1
TA3	50	0.725	0.1813	0.1
TA4	25	0.3625	0.0906	0.1

Figure 4. A) Designed TA–$FeCl_3$–FITC coating scheme and dosage conditions used for fluorescence-based characterization. FITC was incorporated to fluorescently label the tannic acid-based coating, enabling quantification of coating presence across formulation conditions.

Results

Preliminary Results

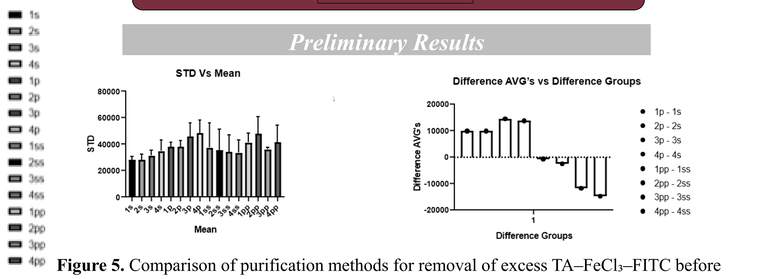


Figure 5. Comparison of purification methods for removal of excess TA–$FeCl_3$–FITC before fluorescence-based coating quantification. Ultracentrifugation-only conditions (s, p) were less consistent than samples with an added tabletop spin (ss, pp), suggesting improved removal of unbound reagents and more reliable measurement of coating associated with liposomes or EVs.

Biochemical Analysis

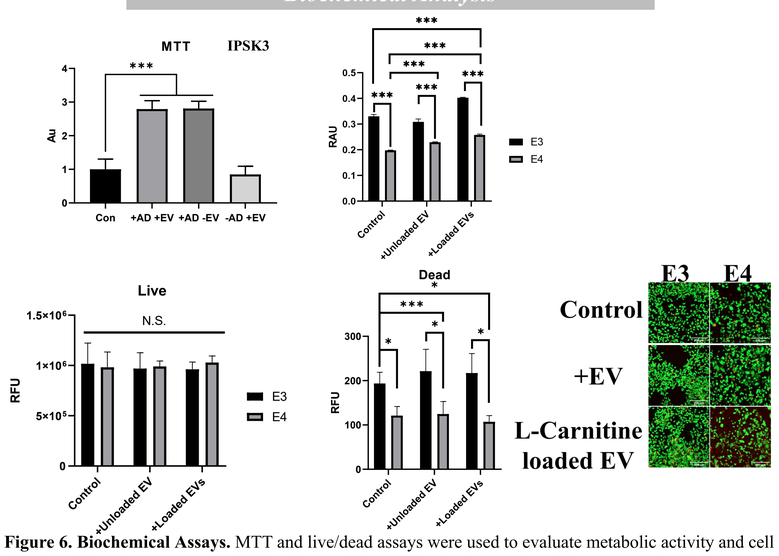


Figure 6. Biochemical Assays. MTT and live/dead assays were used to evaluate metabolic activity and cell viability following treatment with healthy and L-carnitine-loaded EVs, while comparative fluorescence imaging was used to assess treatment-related cellular responses between E3 and E4 groups.

3. EV general characterizations

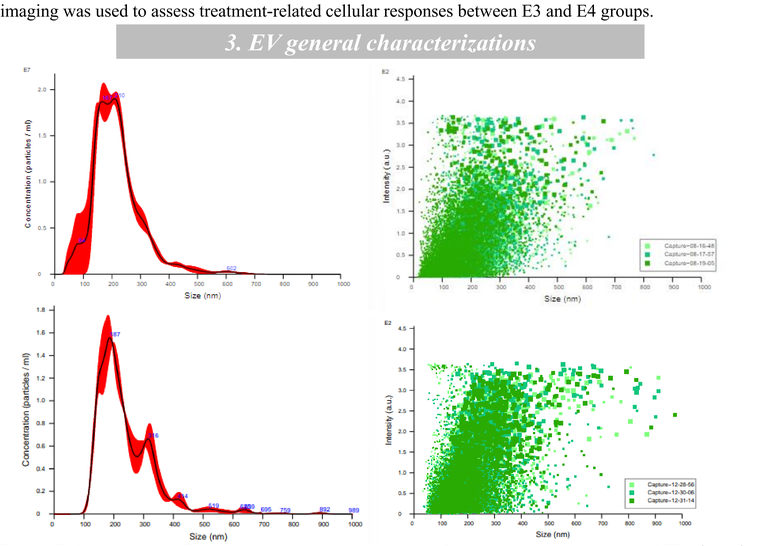


Figure 7. Nanoparticle Tracking Analysis and imaging of exosomes and liposomes. NTA data size comparison of electroporated vs non and cell concentration

Future Work with Tannic Acid Coating

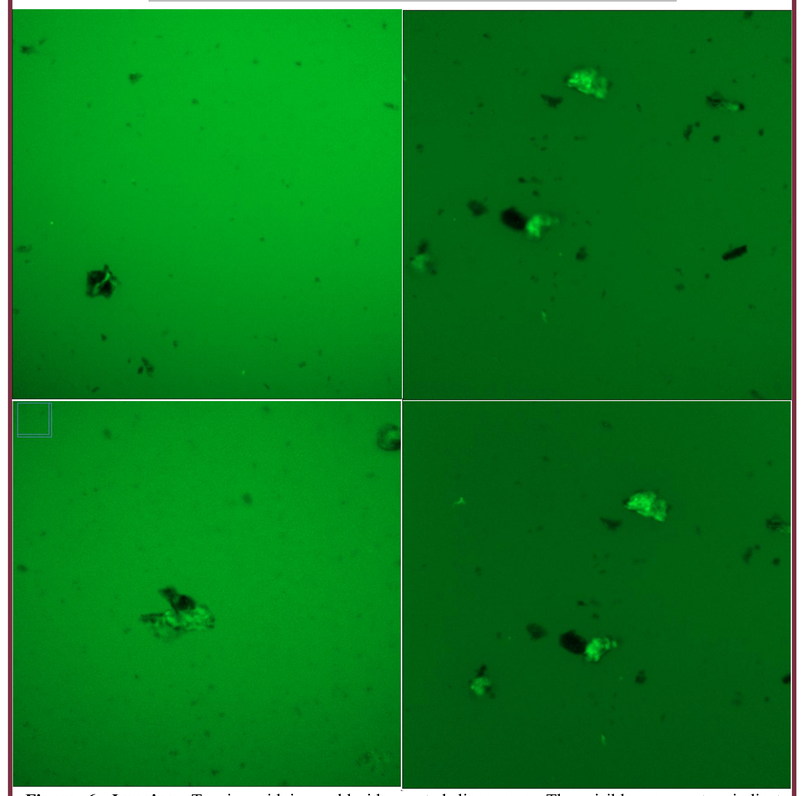


Figure 6. Imaging. Tannic acid–iron chloride coated liposomes. The visible aggregates indicate successful coating formation; however, excess unbound reagents remain present in the system. Optimization of purification to remove residual tannic acid and iron chloride is therefore identified as a future work objective beyond the current scope of this study.

Conclusions

This study established a preliminary framework for evaluating extracellular vesicle-based therapeutic platforms in an Alzheimer's disease model while also developing a surface functionalization strategy for future vesicle engineering. Initial biochemical findings showed that EV treatment influenced cellular response, supporting the idea that vesicle composition and cargo can affect biological behavior in disease-relevant cells. In parallel, synthetic liposomes provided a controlled and reproducible model for developing tannic acid–iron chloride coatings before translating the method to biologically derived EVs, allowing surface modification behavior to be assessed with reduced biological variability. Preliminary imaging confirmed that coating formation was achieved, demonstrating the feasibility of this approach for modifying vesicle-like systems, although excess unbound reagents remained present and indicated that purification optimization is still needed. Overall, these results support continued development of coated vesicle platforms for Alzheimer's disease research and provide a foundation for future work focused on improving coating quantification, refining purification efficiency, and incorporating therapeutic cargo into engineered EV-inspired delivery systems.

References:

Guo et al. 2021

Zhang et al. 2020

Zhao et al. 2020