Targeting Tau Protein Using Ginger-Derived Nanovesicles

Grant Proposal

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Abstract

People with neurodegenerative diseases, which can lead to dementia, have trouble performing everyday tasks. Alzheimer's disease (AD) is a neurodegenerative disorder defined by the accumulation of amyloid-beta plaques and neurofibrillary tangles, contributing to cognitive decline and neuronal dysfunction. Recent research has focused on plant-derived nanovesicles, particularly ginger-derived nanovesicles (GDNVs), as a novel platform for drug delivery to the brain. GDNVs are membrane-bound particles, showing biocompatibility and low immunogenicity. Ginger (*Zingiber officinale*) is an anti-inflammatory rhizome with antioxidant properties containing bioactive molecules like 6-gingerol and 6-shogaol, both can reduce neuroinflammation and oxidative stress—two key contributors to AD pathogenesis (Matin et al., 2024). This project investigates the potential of GDNVs as a targeted drug delivery system for AD. Preliminary locomotion assay data from a wildtype strain of *C. Elegans* (N2) showed a significant increase in speed, but more data is needed to get conclusive results. These results highlight the potential of GDNVs to improve the treatment of Alzheimer's disease by enhancing neuroprotection and slowing disease progression.

Keywords: Alzheimer's disease, Neurodegeneration, Ginger-derived nanovesicles (GDNVs), Neuroinflammation, Therapeutic nanovesicles, Plant-derived nanovesicles, Dementia

Targeting *Tau* Protein Using Ginger-Derived Nanovesicles

Neurodegenerative disorders are among the most challenging health issues, and Alzheimer's disease (AD) represents a sizable part of this burden and affects 6.9 million Americans aged 65 and older (Rajan et al., 2021). These conditions, marked by progressive nervous system degeneration, are increasingly common in the aging population (Thorpe & Woodruff, 2021). Alzheimer's disease diminishes cognitive abilities and significantly impairs daily functioning (Goedert, 2004). The main causes of AD are attributed to beta-amyloid plaques and tau protein tangles: the formation of amyloid plaques amplifies tau tangles (Bloom, 2014). Although considerable research has been conducted, current therapies for AD primarily address symptoms rather than targeting the disease's underlying mechanisms, showing an urgent need for innovative approaches that focus on core pathological features, such as amyloid plaques and tau protein accumulation (Lahiri et al., 2013). Despite an emphasis on amyloid plaques, emerging evidence suggests that tau pathology correlates more directly with cognitive decline and may play a central role in the progression of Alzheimer's disease, highlighting the need to target tau (Holtzman et al., 2016; Bloom, 2014).

Alzheimer's Disease and Tau Protein

Neurofibrillary tangles (NFTs), which are mainly made up of tau protein, are one of the primary indicators of Alzheimer's disease (Goedert, 1996). Under normal conditions, tau binds to and stabilizes microtubules associated with intracellular transport and neuronal structure, promoting axonal integrity and effective transport between neurons (Huang, 2009). However, in AD, tau protein undergoes hyperphosphorylation, where it detaches from microtubules and aggregates into harmful fibrillary tangles within neuron cell bodies and dendrites as seen in Appendix 1 (Jie et al., 2021). This is a process that leads to cellular dysfunction and neuronal death (Wood et al., 1986).

NFTs contribute to AD progression by furthering chronic neuroinflammation, which exacerbates tau pathology and neuronal damage. The accumulation of NFTs activates microglia and astrocytes, leading to the release of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which further promote tau hyperphosphorylation and aggregation (Maccioni et al., 2009; Chen & Yu, 2023). Additionally, tau pathology induces necroptosis through RIPK1 and RIPK3 signaling, amplifying neuroinflammation and neuronal loss (Caccamo et al., 2017).

This abnormal accumulation of tau within NFTs strongly correlates with the severity of AD symptoms and cognitive decline, where targeting hyperphosphorylated tau can provide the quickest therapeutic target (Guha et al., 2020). The accumulation of hyperphosphorylated tau into NFTs is significantly associated with the severity of AD symptoms, as evidenced by tau-induced mitochondrial stress responses showing highly significant differences ($p < 0.001$), highlighting its potential as a therapeutic target (Guha et al., 2020). Hyperphosphorylated tau not only impairs axonal transport but also disrupts normal cytoskeletal interactions, causing neuronal instability and further neurodegenerative progression. Despite advances in tau-targeted therapies, such as anti-tau monoclonal antibodies (like aducanumab© and gantenerumab©) and tau aggregation inhibitors (such as LMTX©), An effective and safe method to deliver these treatments remain a significant challenge in AD research (Congdon et al., 2023).

Ginger Derived Nanovesicles (GDNVs)

Recently, plant-derived nanovesicles, such as ginger-derived nanovesicles (GDNVs), have been discovered as a novel approach for delivering therapeutic agents targeting AD related pathology. These naturally occurring, membrane-bound particles have come to the spotlight as a potential method to deliver therapeutic molecules directly to diseased tissues (Butreddy et al., 2021; Yu et al., 2020). Plant-derived nanovesicles, particularly those from ginger, offer multiple advantages: they are biocompatible and show low immunogenicity. These properties allow GDNVs to potentially be a more efficient, targeted system for drug delivery in AD (Yu et al., 2020). These particles are also already widely used and are relatively cheap to extract. This could provide for a cost-effective method compared to the expensive methods used today.

Ginger is a source of potent anti-inflammatory and antioxidant compounds, like 6-gingerol and 6-shogaol, which helps reduce inflammation — key factors implicated in AD pathology (Ballester et al., 2022; Akiyama, 2000). These compounds are known to inhibit pathways associated with neuroinflammation and oxidative stress, including NF-kB and COX-2, demonstrated to be elevated in AD brains and can lead to disease progression (Wang et al., 2014). Studies have shown that these bioactive compounds can slow the release of pro-inflammatory cytokines that cause inflammation like TNF-α and IL-1β, reducing neuronal damage (Ballester et al., 2022).

Ginger Ingredient	Hallmark of Aging	In Vitro Model and Reference	Dose and Time of Treatment	Outcomes
8-Paradol	Chronic inflammation	COX-1 inhibitor assay [41]	Tested for 10 min in several concentrations (0–100 μM) for IC50 determination	Among several ginger-derived compounds, 8-paradol exhibited the strongest COX-1 inhibitory activity, with an IC50 value of 4 μM
10-Gingerol, 8-shogaol and 10-shogaol	Chronic inflammation	COX-2 inhibitor assay [42]	Each compound was tested in 12 different concentrations for IC50 determination	The three compounds inhibited COX-2 with IC50 values of 32 μM, 17.5 μM, and 7.5 μM, respectively
Steamed ginger extract	Chronic inflammation	Helicobacter pylori-infected gastric (AGS) cells [44]	Applied at 1–200 μg/mL for 24 h	Inhibited the growth of Helicobacter pylori, but also reduced in the gastric cells Helicobacter pylori-induced inflammation markers, including IL-8, TNF-α, IFN-γ, IL-6, iNOS, and myeloperoxidase
6-Shogaol	Chronic inflammation	LPS-challenged HUVECs [45]	Applied 30 min before LPS challenge at 1–30 μM [45]	Exhibited inhibitory effects on angiogenesis and inflammation in LPS-activated HUVECs, inhibiting the activation of NF-κB, the expression of pro-inflammatory adhesion molecules (ICAM-1, VCAM-1, E-selectin), and the attachment of leukocytes
6-Shogaol	Chronic inflammation	Rats subjected to monoarthritis in the right knee by Freund's Adjuvant injection [46]	Applied daily via gavage at 6.2 mg/kg for 28 days	Decreased chronic inflammatory response and provided protection against damage to femoral cartilage, reflected in reduced swelling, preservation of the morphological integrity of the cartilage lining the femur, reduced leukocyte infiltration, and lower concentration of soluble 1 VCAM-1

Table 1: From Matin et al. (2024). This table shows the ingredients in ginger that affect Chronic inflammation when studied in vitro. This study shows that these ingredients in ginger inhibited inflammation by either inhibiting compounds leading to inflammation or directly targeting the inflammation.

Specifically, Matin et al. (2024) showed in their in-vitro studies that 6-shogaol and other ingredients in ginger could inhibit inflammation. These bioactive molecules present in GDNVs may slow cognitive decline and protect neuronal health in AD.

GDNVs are promising due to their ability to carry and protect bioactive compounds from premature degradation, improving stability and bioavailability. Research has indicated that ginger's bioactive compounds, when delivered effectively, may not only help mitigate inflammatory responses but also improve antioxidant defenses within the brain, potentially modulating pathways involved in apoptosis and autophagy that are critical in AD cell survival and maintenance (Yu et al., 2020). As such, the integration of ginger-derived nanovesicles into AD therapies could lead way to a multi-targeted approach to slowing or altering disease progression. Further leading to enhanced protection of neural cells and reduction of neurotoxic effects associated with oxidative damage and protein misfolding (Marban & Grigorian, 2023; Ballester et al., 2022).

Tau Aggregation Targeting

This research aims to address these challenges by investigating whether GDNVs can effectively deliver tau-targeting agents to mitigate tau hyperphosphorylation in AD. Given the pathological significance of tau in AD and the promising bioactivity and delivery properties of GDNVs, their combination offers a compelling therapeutic approach. By delivering treatments such as tau aggregation inhibitors directly to affected brain regions, GDNVs may enable a more precise approach to disease modification (Yin et al., 2023).

It is hypothesized that GDNVs can be an effective delivery system for tau-targeting therapies, enabling targeted action within neurons and mitigating neurodegeneration in AD. To test this, GDNVs will be isolated, and loaded into *C. elegans* with aggregated tau, and evaluate their effects on the tau concentration in vivo. The process

will involve GDNV isolation, characterization, and encapsulation of tau-targeting agents (Novo et al., 2022). Through this process, the therapeutic potential of GDNVs in addressing tau-related neurotoxicity, cellular damage, and cognitive decline in AD can be evaluated.

Relevance

This research could contribute to a new, biocompatible, and cost-effective method of targeting tau pathology in AD and could have similar contributions to other tauopathies or neurodegenerative disorders. A successful GDNV-based delivery system would expand the utility of nanovesicle technology, providing a sustainable, accessible, and scalable therapeutic solution (Sapan et al., 1999). This approach could target one of the root causes of neurodegeneration, significantly advancing the field of neurodegenerative disease research.

Section II: Specific Aims

This project aims to develop and evaluate ginger-derived nanovesicles (GDNVs) as a tau-targeting agent to mitigate tau hyperphosphorylation, neurofibrillary tangles, and neurotoxicity in Alzheimer's disease (AD). The rationale is that if GDNVs display tau-suppressing properties, they can be further developed for humans with AD-caused dementia.

Specific Aim 1: Isolation of GDNVs

The first aim focuses on the isolation and detailed characterization of ginger-derived nanovesicles (GDNVs) develop a reproducible preparation process suitable for therapeutic applications. GDNVs will be isolated through a systematic protocol. Achieving this aim will ensure that the GDNVs are consistently prepared and fit for use in therapeutic studies targeting tau pathology in Alzheimer's disease.

Specific Aim 2: Targeting of Tau Suppressing Agents into *C. Elegans*

The second aim is to test the therapeutic potential of GDNVs in a *C. elegans* model of tau aggregation. Two strains of *C. elegans* will be used: BR5270 (pro-aggregation) and BR5271 (anti-aggregation). These strains will be cultured on nematode growth medium (NGM) plates seeded with *E. coli* OP50 and incubated at 20°C to keep healthy populations (Konietzka et al., 2019).

The anti-inflammatory and neuroprotective effects of GDNVs will be confirmed using two main approaches:

Fluorescence Microscopy

Worms expressing mCherry-tagged tau will be observed under a fluorescence microscope to quantify tau aggregates. After incubation, worms will be washed with M9 buffer to remove residual GDNVs. Fluorescent imaging will focus on areas of the worm where tau aggregation is most pronounced, such as the head or body region. A FlyPi will be used to perform this fluorescence imaging (Chagas et al., 2017). Quantitative imaging analysis will calculate fluorescence intensity to measure changes in tau levels between treated and control groups.

Behavioral Observations

General health and mobility will be monitored daily using a dissecting microscope. Differences in movement, speed, and overall vitality between treated and untreated groups will be recorded to evaluate the potential functional benefits of GDNV treatment.

Expected Outcome

The successful isolation and utilization of GDNVs will open a new avenue for treatment of tau aggregation and neurotoxicity associated with Alzheimer's disease. These findings may extend the application of other plant-derived nanovesicles in other neurodegenerative disorders, thus offering promise for neurotherapeutics.

Section III: Project Goals and Methodology

Relevance/Significance

Alzheimer’s disease (AD) affects millions worldwide, with a prevalence that is expected to rise as populations age (Rajan et al., 2021). Despite decades of research, available treatments focus on managing symptoms rather than focusing on the core pathological mechanisms, such as tau protein aggregation and neuroinflammation (Lahiri et al., 2013). Tau hyperphosphorylation and the formation of neurofibrillary tangles (NFTs) are strongly correlated with the disease’s severity, making tau a key therapeutic target (Goedert, 1996; Wood et al., 1986).

The focus is ginger-derived nanovesicles (GDNVs) as a delivery platform for tau-targeting therapies addresses the urgent need for novel and effective treatment strategies. GDNVs are biocompatible, show low immunogenicity, and are derived from a sustainable natural source, making them a great alternative to synthetic delivery systems (Yu et al., 2020). Furthermore, the bioactive compounds in ginger, such as 6-gingerol and 6-shogaol, have proven anti-inflammatory and antioxidant properties that may synergize with tau-suppressing agents

to reduce neuroinflammation and oxidative stress—both critical contributors to AD progression (Ballester et al., 2022; Wang et al., 2014).

By exploring GDNVs for therapeutic delivery, this research contributes to the growing field of plant-derived nanotechnology and offers a novel approach to addressing the root causes of AD pathology. If successful, this work could significantly advance therapeutic options for AD and other neurodegenerative diseases, potentially optimizing treatment on a global scale.

Innovation

This project introduces plant-derived nanotechnology for tau-targeting therapy for Alzheimer's disease. Ginger-derived nanovesicles (GDNVs) are a unique drug delivery system that leverages the natural bioactivity of ginger, including its anti-inflammatory and antioxidant compounds, alongside the vesicles' structural and functional properties (Yu et al., 2020; Ballester et al., 2022). Unlike synthetic nanoparticles, GDNVs are sustainable, cost-effective, and show minimal toxicity, making them great for therapeutic applications in neurodegenerative diseases.

A key innovative aspect of this experiment is the targeting of hyperphosphorylated tau protein in *C. elegans* models of tauopathy. Using *C. elegans* as a model organism allows for better analysis of tau aggregation, neurotoxicity, and behavioral changes, providing a streamlined yet effective experimental framework (Ruszkiewicz et al., 2018; Roussos et al., 2023).

Another innovative feature is the application of advanced imaging techniques, such as fluorescence microscopy, to quantify tau aggregation in vivo, paired with behavioral assays to test functional outcomes (Wood et al., 1986; Yin et al., 2023). This evaluation provides a detailed assessment of GDNV efficacy, leading the way for translational applications in other animal models and, eventually, clinical settings.

Methodology

Specific Aim #1: Isolation of GDNVs

The ginger root will be juiced in a blender with sterile water to obtain a homogenous mixture. The juice is filtered through a fine mesh or cheesecloth to clear the pulp and larger particulates. Centrifugation will be carried out on filtered juice for the isolation of GDNVs. Initial centrifugation at 3000 x g for 10 minutes will remove cellular debris and larger particles. The solution then undergoes ultracentrifugation at 100,000 x g for 1–2 hours,

during which time the GDNVs will pellet. The pellet must be washed with PBS to remove residual impurities. Finally, the GDNV pellet will be resuspended in PBS, quantified, and divided into desired concentrations (10–100 μg/mL). Prepared GDNVs will be stored at 4°C for short-term use to preserve integrity and function (Chen et al., 2024).

Justification and Feasibility. Ginger-derived nanovesicles have shown promise as natural, biocompatible carriers with anti-inflammatory properties. Prior research supports the feasibility of isolating nanovesicles from plant sources using similar protocols. Liu et al. (2020) showed that differential centrifugation effectively isolates plant-derived nanovesicles with high purity, keeping their structural integrity. Figure 1 shows leaf nanovesicles used to show a consistent size and shape after isolation by differential centrifugation. The images show both sEVs and leaf nanovesicles possess a single lipid bilayer (Liu et al., 2020). Further, the nanoparticle tracking analysis (NTA) results confirm the TEM images, providing insight into the overall size uniformity of vesicle preparations. The average zeta potential for leaf nanovesicles and sEVs are like those of mammalian sEVs and some other plant-derived sEV-like nanovesicles (Liu et al., 2020). The reproducibility of this method guarantees that vesicles were prepared with uniformity in size and composition, needed for therapeutic targets. Ginger would create a biologically active base for nanovesicle-based therapies. Adopting this protocol ensures reproducibility, enabling the mass production of GDNVs for subsequent therapeutic investigations based on antioxidant and anti-inflammatory effects due to the presence of the constituents 6-gingerol and 6-shogaol.

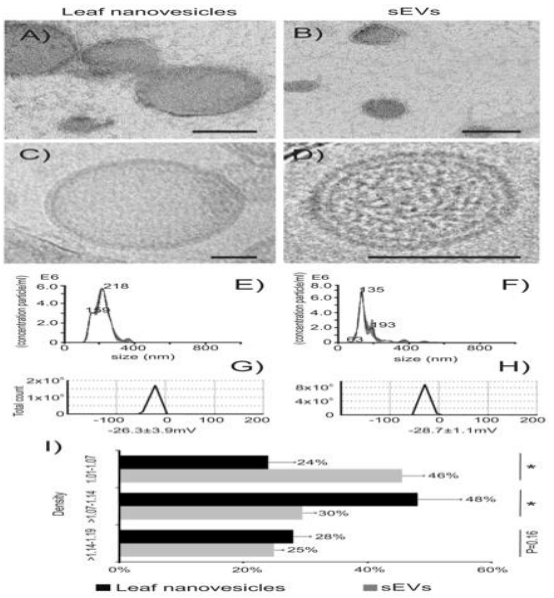

Figure 1: From Liu et al. (2020). Characterization of leaf nanovesicles and sEVs. (A, B) TEM images leaf nanovesicles and sEVs (bar = 100 nm). (C, D) Cryo-EM image of leaf nanovesicles and sEVs (bar = 50 nm). (E, F) Leaf nanovesicle and sEVs size distributions, as assessed by nanoparticle tracking analysis. (G, H) Zeta potential of leaf nanovesicles and sEVs. (I) The protein distributed over the sucrose density fractions of the leaf nanovesicles (black bars) and sEVs (gray bars); mean value is shown in the graph.

Summary of Preliminary Data. Preliminary experiments successfully isolated GDNVs using the outlined method. These findings confirm the method's reliability and the GDNVs' potential for downstream applications.

Expected Outcomes. This aim is to set up a reproducible method for isolating high-quality GDNVs, ensuring their structural and compositional integrity. The isolated GDNVs will be a reliable platform for therapeutic applications, particularly in tau pathology studies.

Potential Pitfalls and Alternative Strategies. Challenges may include variability in GDNV yield due to differences in ginger sources or contamination with non-vesicular components. These issues will be avoided by optimizing the centrifugation parameters and using added filtration or density gradient centrifugation steps. If variability occurs, alternative ginger sources or pre-treatment processes may be used.

Specific Aim #2: Targeting of Tau Suppressing Agents into C. elegans

This aim will evaluate the therapeutic potential of ginger-derived nanovesicles (GDNVs) encapsulating tau-targeting agents in a *C. elegans* model of tau aggregation. Two strains of *C. elegans* will be employed: BR5270 (pro-aggregation) and BR5271 (anti-aggregation). These strains are well-suited for this experiment (DeVos et al., 2017). Both strains will be cultivated on nematode growth medium (NGM) plates seeded with E. coli OP50 as a food source and kept at 20°C T\to guarantee healthy populations (Park et al., 2017; Virk et al., 2016; Konietzka et al., 2019). Experimental groups will be treated with GDNVs at 10, 50, and 100 µg/mL to test dose-dependent effects, while control groups will receive no treatment. GDNV suspensions will be added directly to the growth medium for uniform exposure, and the worms will be incubated for 24–48 hours. Tau pathology and therapeutic outcomes will be evaluated using fluorescence microscopy and behavioral observations. Fluorescent imaging will quantify tau aggregates in *C. elegans* expressing mCherry-tagged tau, focusing on regions of high aggregation, while behavioral assays will monitor motility, speed, and vitality to test functional outcomes.

Justification and Feasibility. The use of *C. elegans* as a model for neurodegenerative diseases provides a cost-effective and well-established system for studying tau pathology and evaluating potential therapeutic agents. This nematode offers a simple, highly conserved nervous system, allowing for the investigation of molecular mechanisms underlying tau aggregation while facilitating translational research. Furthermore, a short lifecycle and ease of genetic manipulation make *C. elegans* an ideal organism for high-throughput studies. The strains chosen for this experiment, BR5270 and BR5271 from the Caenorhabditis Genetics Center (CGC), have been validated in previous research as reliable models for tau aggregation and suppression, respectively, ensuring the relevance of the experimental design (DeVos et al., 2017).

The use of ginger-derived nanovesicles (GDNVs) to deliver tau-targeting agents leverages the vesicles' biocompatibility and bioactive properties, as demonstrated in prior studies on their anti-inflammatory and antioxidant effects. This aim is further supported by the availability of fluorescence microscopy for quantifying tau

aggregates and behavioral assays for assessing functional improvements. Evaluating different GDNV concentrations (10, 50, and 100 µg/mL) can show dose-dependent effects, improving the significance of the experiment's findings. In this regard, the experiment is feasible in terms of its aim and offers significant insight into the therapeutic values of GDNVs for treating tau pathology.

Summary of Preliminary Data. The data showed that treated C. Elegans showed signs of faster locomotion. As shown by figure 3, the speeds of the worms vary significantly. Treated worms had a much higher speed (an average of 163.46 µm/s) compared to the untreated worms which averaged 92.55 µm/s. When a t-test for difference in means was conducted, the p-value was 1.4669 x 10^-14, showing that this result was promising and very significant. However, as figure 4 shows, the test for body bends showed that there was no significant difference between the two groups. The treated group showed 16.78 waves/min while the untreated group showed 16.89 waves/min. The p-value for this test was 0.83558 and showed that there was no significant difference between the groups. Locomotion speed and body bend assays are good ways to assess tau protein levels as they affect AD. AD also causes slower movement speeds and reduced locomotion in both *C. elegans* and humans, which is why *C. elegans* are an optimal model for this experiment (Alvarez et al., 2022).

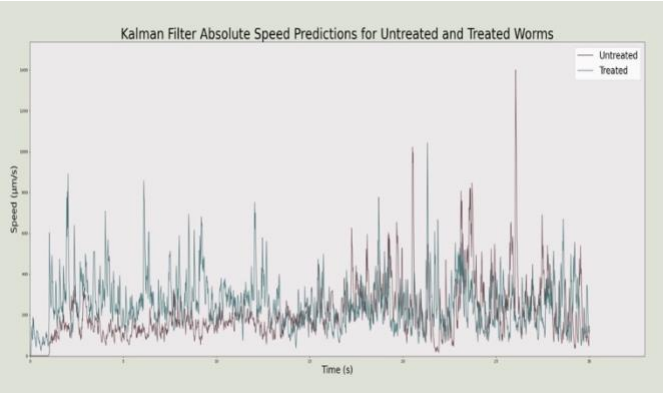

Figure 3: The speeds of 24 worms fed through a Kalman Filter to reduce noise graphed for 40 seconds. There is a clear difference between the treated and untreated worms' speeds.

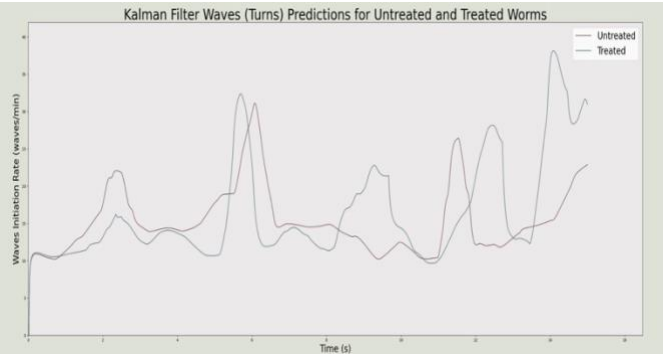

Figure 4: Worms that were approximately the same size were selected from each sample. Data from WormLab. The average wave/min was then fed into a Kalman Filter to smooth out any noise or random errors in the data.

Preliminary studies using plant-derived nanovesicles, including those from ginger, have shown promising anti-inflammatory and neuroprotective effects in various disease models (Yu et al., 2020; Ballester et al., 2022). In tauopathy models, similar interventions have been shown to reduce tau aggregation and improve neuron health (Santacruz et al., 2005; Xu et al., 2014; DeVos et al., 2017).

Expected Outcomes. The experiments are expected to show a dose-dependent reduction in tau aggregation in BR5270 worms treated with GDNVs encapsulating tau-suppressing agents. Specifically, fluorescence microscopy

should reveal a decrease in mCherry-tagged tau aggregates in the head and body regions of treated worms, with the highest dose (100 µg/mL) producing the most pronounced effect. Behavioral assays are anticipated to demonstrate improvements in motility, with treated worms displaying faster movement, more frequent turns, and increased vitality compared to untreated controls.

In addition to tau suppression, it is expected that GDNV treatment will exhibit neuroprotective effects, possibly through the anti-inflammatory properties of ginger-derived bioactive compounds. These benefits, as demonstrated by Matin et al. in 2021, may include reduced oxidative stress and better maintenance of neuronal integrity in the worms. Collectively, the results will provide compelling evidence of the therapeutic potential of GDNVs as a novel delivery system for combating tauopathies.

Potential Pitfalls and Alternative Strategies. One potential challenge is that GDNVs may not effectively encapsulate or deliver tau-suppressing agents in sufficient quantities to produce a therapeutic effect. Encapsulation protocols could be optimized by altering GDNV preparation conditions or employing alternative agents with higher affinity for tau. Additional encapsulation strategies, such as incorporating nanoparticles or lipophilic enhancers, may be tested to improve drug delivery efficiency ff the expected reduction in tau aggregation is not observed.

Section IV: Resources/Equipment

The resources for this experiment include *C. elegans* tau protein strains. Specifically, the BR5270 strain (pro-aggregation) and the BR5271 strain (anti-aggregation) will be used, with both strains being requested from the Caenorhabditis Genetics Center (CGC). Fresh ginger root will be used for GDNV extraction and phosphate-buffered saline (PBS) will be used for washing *C. elegans* during the experimental process.

General laboratory supplies that are needed include petri dishes for culturing and treating *C. elegans*, pipette tips in varying sizes for accurate liquid handling, and microcentrifuge tubes (1.5 mL and 15 mL) for sample preparation and protein extraction. Filter paper will also be required to strain ginger juice during the extraction process. A blender for ginger extraction, a centrifuge for the initial step of sample purification (3000 x g for 10 minutes), and an ultracentrifuge for pelleting the GDNVs (100,000 x g for 1–2 hours) will also be needed. Finally, a fluorescence microscope (specifically a FlyPi) will be used to observe and quantify mCherry-tagged tau aggregates in *C. elegans*.

A FlyPi is a low-cost, fluorescence-based microscope based on a Raspberry Pi (Chagas et al., 2017). FlyPi is well-fitted for this project as it has advanced staining capabilities for fluorescence microscopy. The platform has other excellent specifications for imaging fluorescently labeled samples like the mCherry-tagged tau aggregates in the *C. elegans* model, which can be quantified and measured for tau pathology. The FlyPi system is designed with specialized camera software to provide accurate and detailed images, which is crucial for evaluating the effects of GDNVs on tau aggregation in neurodegenerative disease models (Chagas et al., 2017).

Section V: Ethical Considerations

This experiment adheres to ethical principles, particularly regarding the use of *C. elegans* as a model organism. These nematodes are cultured in controlled environments to ensure optimal living conditions, including appropriate temperature, feeding, and handling. This includes autoclaving the NGM and using 10% bleach to dispose of the worms. Efforts are made to minimize stress, avoid unnecessary suffering, and use the smallest number of worms required to achieve robust, statistically significant results. This approach aligns with the ethical principles outlined by Replacement, Reduction, and Refinement (3Rs): Replace the animals if possible; if not, reduce the number of animals used; and lastly, minimize the suffering of animals used of studies (The 3Rs, n.d.). Although *C. elegans* is widely accepted for high-throughput studies due to its simplicity and rapid lifecycle, its use in studying human disease mechanisms necessitates thoughtful justification, which this study provides by focusing on tau aggregation, a critical feature of Alzheimer's disease.

Additionally, the extraction and application of ginger-derived nanovesicles (GDNVs) in the experiment pose minimal ethical concerns. Ginger is a plant material sourced sustainably, and its use does not involve harm to animals or the environment. The goal of the research further justifies the ethical use of *C. elegans* and plant-derived materials.

Section VI: Timeline

The study will span 1–2 months, beginning with the preparation of materials, including *C. elegans* strains (BR5270 and BR5271), fresh ginger for GDNV isolation, and necessary reagents like PBS and M9 buffer. In the first week, GDNVs will be extracted using a juicing and centrifugation, while *C. elegans* will be cultured for healthy growth. Baseline fluorescence microscopy and behavioral observations will be conducted in Week 2 to establish tau aggregation and motility in untreated groups. In Weeks 3 and 4, *C. elegans* will be treated with varying GDNV

concentrations (10, 50, and 100 μg/mL) and incubated for 24–48 hours, followed by fluorescence imaging to quantify tau aggregates and behavioral assays to monitor vitality and motility. Week 5 will focus on analyzing fluorescence and behavioral data to evaluate therapeutic effects, and the final week will involve compiling results, visualizing data, and drafting a comprehensive report on the potential of GDNVs in mitigating tau pathology.

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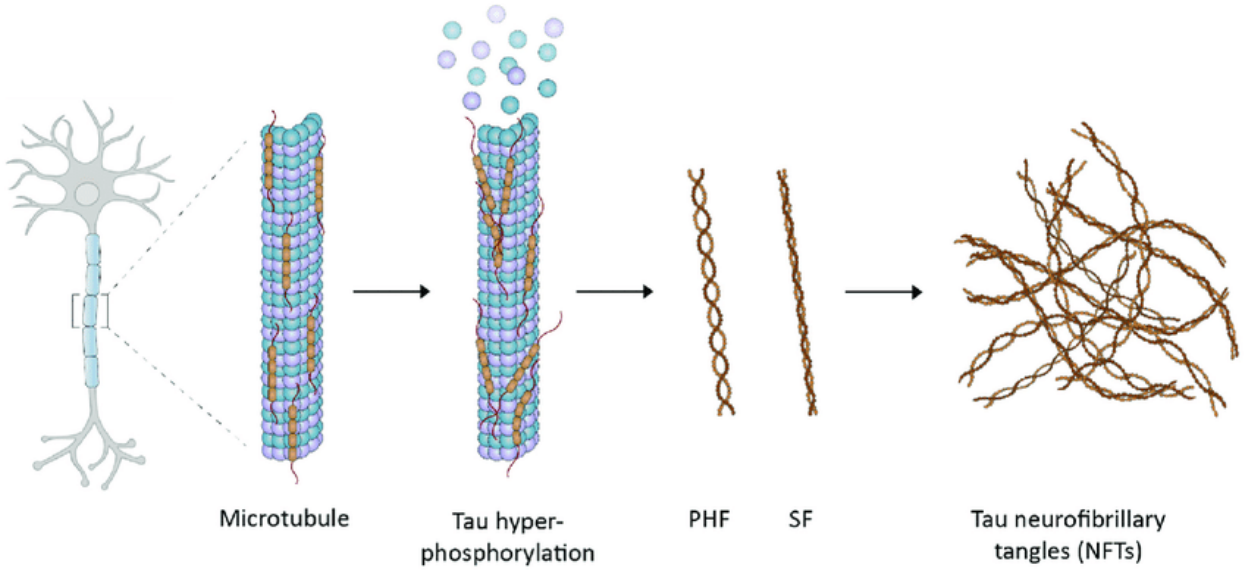
Section VIII: Appendix

Appendix 1: Structure of NFTs



Appendix 1: Progression of the formation of NFTs taken from Jie et al., 2021. Tau proteins aggregate and form loose intertwined and tightly intertwined structures which lead to NFT formation.