Review Article

Cell culture research in aging and Alzheimer's disease: The strategic use/reuse of untreated controls and savings people's tax dollars

Journal of Alzheimer's Disease Reports
Volume 9: 1–18

DOI: 10.1177/25424823241310716
journals.sagepub.com/home/alr

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Abstract

Cell culture is an essential tool in both fundamental and translational research, particularly for understanding complex diseases like Alzheimer's disease (AD). The use of cell lines provides the advantage of genetic homogeneity, ensuring reproducible and consistent results. This article explores the application of mammalian cell cultures to model AD, focusing on the transfection of cells with key genes associated with the disease to replicate the cellular environment of AD. It explains various transfection methods and challenges related to the process. These models offer a robust platform for investigating cellular biology, molecular pathways, physiological processes, and drug discovery efforts. A range of assays, including RT-PCR, western blotting, ELISA, mitochondrial respiration, and reactive oxygen species analysis, are employed to assess the impact of genetic modifications on cellular functions and to screen potential AD therapies. Researchers often design experiments with multiple variables such as genetic modifications, chemical treatments, or time points, paired with positive and negative controls. By using a consistent control group across all conditions and under identical experimental conditions, researchers can minimize variability and enhance data reproducibility. This approach is particularly valuable in AD research, where small experimental differences can significantly influence outcomes. Using a shared control group ensures data comparability across experiments, saving time and resources by eliminating redundant control tests. This strategy not only streamlines the research process but also improves the reliability of results, making it a sensible, resource-efficient method that ultimately conserves public funding in the pursuit of AD treatments.

Keywords

Alzheimer's disease, amyloid-β protein precursor, cell culture, enzyme-linked immunosorbent assay, induced pluripotent stem cells, neuronal cell lines, transfection

Received: 10 October 2024; accepted: 4 December 2024

Introduction

Cell culture is a foundational technique in biomedical research, enabling the in vitro study of cellular processes in a controlled environment. This method involves the growth and maintenance of cells derived from multicellular organisms, allowing researchers to investigate cellular behaviors, drug responses, and molecular pathways often challenging to study in vivo. Cell culture systems have evolved significantly since their inception, with advancements in culture conditions, media formulations, and cell lines that have expanded their applications in various fields, including cancer research, drug development, and regenerative medicine.

In the context of biomedical research, cell culture provides a versatile platform for dissecting complex biological mechanisms at the cellular and molecular levels. It allows

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for the manipulation of the extracellular environment, enabling the study of specific variables such as nutrient availability, oxygen levels, and the presence of signaling molecules, which can influence cell behavior and fate. Moreover, cell culture models serve as critical tools for preclinical drug testing, offering a preliminary assessment of a compound's efficacy and toxicity before advancing to animal models and human clinical trials (Figure 1).[1,2]

Despite its widespread use, cell culture is not without limitations. One of the primary challenges is replicating the in vivo environment, as cells in culture often lack complex interactions with other cell types and the extracellular matrix that they would experience in a living organism. Additionally, prolonged culture can lead to genetic and phenotypic drift, potentially altering the cells' behavior and responses over time.[3] Nonetheless, ongoing advancements in three-dimensional culture systems, co-culture techniques, and organ-on-a-chip technologies are helping to address these limitations, bringing cell culture models closer to mimicking physiological conditions.

In conclusion, cell culture remains an indispensable tool in biomedical research, offering unparalleled insights into cellular functions and disease mechanisms. As technologies continue to advance, the accuracy and relevance of cell culture models will only improve, further solidifying their role in the future of biomedical research.

The purpose of this article is to discuss the use/reuse of the same untreated control group in experiments, highlighting its importance in reducing variability, enhancing data robustness, and saving resources. By using consistent controls, the study emphasizes the potential to minimize experimental costs, ultimately benefiting research funding efficiency, including saving taxpayers' money through more economical use of public research funds. This approach helps ensure that funds are allocated efficiently for high-quality research while reducing unnecessary duplication of effort and resources.

Cell lines for aging and Alzheimer's disease research

Cell lines play a crucial role in aging and Alzheimer's disease (AD) research, providing models to study the cellular and molecular mechanisms underlying these complex processes. These cell lines offer insights into the pathophysiology of aging and AD, as well as platforms for drug testing and therapeutic development. Below are some key cell lines used in this field (Table 1, Figure 2).

SH-SY5Y cells

Type: human neuroblastoma cell line. Application: Widely used in neurobiology, these cells can be differentiated into neuron-like cells (Figure 3(a)), making them a popular model for studying neurodegenerative diseases, including AD. SH-SY5Y cells express amyloid-β protein precursor (AβPP) and are often used to investigate the processing of AβPP and the formation of amyloid-β (Aβ) plaques, which are hallmarks of AD.[4] Another researcher utilized in vitro $A\beta_{1-42}$ peptide treated differentiated human neuroblastoma SH-SY5Y cell lines as cellular AD model to study mitochondrial viability, oxidative status, and neuronal apoptosis.[5,6] Same SH-SY5Y cell lines treated with $A\beta_{25-35}$ peptide also used to examine mitochondrial oxidative phosphorylation and neurotoxicity.[7] In AD research, transfection is also used to introduce tau protein mutations (e.g., MAPT mutations), which are implicated in neurofibrillary tangle formation, a characteristic feature of AD. For example, transfecting cells with wild-type or mutant tau plasmids can help researchers study tau phosphorylation, aggregation, and the effects of small molecules that inhibit tau aggregation.[8]

M17 cell line

Type: human neuroblastoma cell line. Application: M17 cells (treated with $A\beta_{1-42}$) were also applied to examine the protective effects of DHA and CoQ10, both alone and in combination, against alterations in mitochondrial function caused by Aβ.[9]

N2a cells

Type: mouse neuroblastoma cell line. Application: N2a cells are widely used in neuroscience research, particularly in the study of neurodegenerative diseases like AD. They can be differentiated into neuron-like cells and are often employed to investigate AβPP processing, Aβ production, and the effects of various compounds on neuronal function. N2a cells are valuable for studying the molecular mechanisms underlying amyloid plaque formation, one of the key pathological features of AD.[10] Mouse neuroblastoma (N2a) cells incubated with the Aβ peptide and electron and confocal microscopy, gene expression analysis, and biochemical methods were applied to study Aβ induced changes in mitochondrial structure and function, and neurite outgrowth.[11]

PC12 cell line

Type: mouse neuroblastoma cell line. Application: In vitro AβPP mutant-overexpressed and Aβ peptide-treated PC12 cell lines were used to conduct ATP production assay, MTT assay and reactive oxygen species (ROS)-induced apoptosis assays.[12,13] Decreased mitochondrial membrane potential, the cleavage of poly (ADP-ribose) polymerase, an increase in the Bax/Bcl-X(L) ratio, and activation of

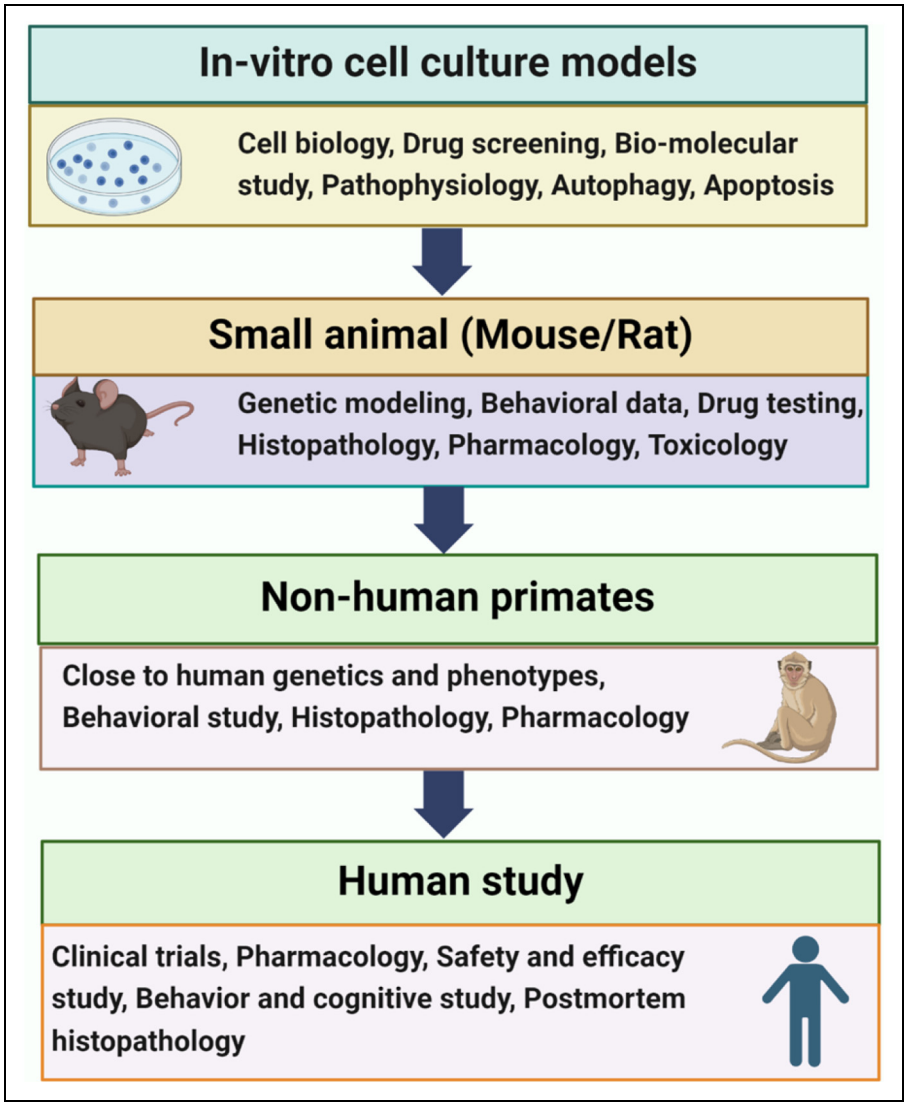

Figure 1. Experimental models in studying the AD pathophysiology ranging from cell biology to clinical trials.

c-Jun N-terminal kinase and oxidative cell death were also reported in a PC12 cell lines after Aβ treatment.[14]

HT22 cells

Type: mouse hippocampal cell line. Application: HT22 cells are derived from the mouse hippocampus and are used as a model for studying oxidative stress and excitotoxicity, both of which are relevant to AD (Figure 3(a)). These cells are particularly useful for investigating the role of glutamate toxicity and oxidative damage in neuronal cell death, processes that are implicated in the neurodegeneration observed in AD.[15] HT22 cells also serve as a model for testing neuroprotective compounds like mitophagy enhancers.[16] Transfection is often employed to knock down genes involved in the antioxidant response, such as Nrf2 (nuclear factor erythroid 2-related factor 2), or to overexpress oxidative stress-inducing proteins, to better understand neurotoxicity and cell death mechanisms. For example, transfecting HT22 cells with plasmids encoding superoxide dismutase (SOD1) or catalase helps investigate the role of oxidative stress in neurodegeneration and the protective effects of antioxidants.[17]

Microglial cell lines (e.g., BV2)

Type: mouse microglial cell line. Application: Microglia are the resident immune cells of the brain, playing a critical role in neuroinflammation, which is a key feature of AD. BV2 cells are commonly used to study microglial activation, cytokine production, and their interactions with amyloid-beta, providing insights into the inflammatory processes in AD.[18] Transfection of these cells with pro-inflammatory cytokine genes or microRNAs

Table 1. Cell lines utilized in aging and Alzheimer's disease.

Sl. no.	Cell line	Origin	Cell type	Applications in AD research
1	SH-SY5Y Cells	Human	neuroblastoma	• synaptic changes • mitochondrial oxidative phosphorylation • neurotoxicity
2	M17 cell line	Human	neuroblastoma	• mitochondrial functions
3	N2a Cells	Mouse	neuroblastoma	• molecular mechanisms underlying amyloid plaque formation • gene expression analysis • mitochondrial structure and function, • neurite outgrowth
4	PC12 cell line	Mouse	neuroblastoma cell line	• ATP production assay • mitochondrial membrane potential • MTT assay • apoptosis assays
5	HT22 Cells	Mouse	Immortalized hippocampal cells	• mitochondrial biogenesis • mitochondrial dynamics • synaptic proteins
6	BV2 cells	Mouse	microglial cell	• Aβ-induced inflammation • microglia activation • cytokines production
7	H4 Cells	Human	neuroglioma	• mechanisms of tau phosphorylation and aggregation • effects of therapeutic compounds on tau pathology • metabolomic and proteomic analysis
8	C6 cells	Rat	Glioma	• apoptosis • intracellular calcium overload generated by $A\beta_{1-40}$ • nitric oxide generation
9	HEK293 Cells	Human	Embryonic kidney cells	• therapeutic compounds screening • intracellular $A\beta_{42}$ aggregation • mitochondrial biogenesis • mitochondrial structure and function
10	IMR-90 cells	Human	Fibroblasts	• role of senescence in neurodegeneration • oxidative stress • apoptosis
11	WI-38 Cells	Human	Fibroblasts	• H_2O_2-induced premature senescence • MTT assay • β-galactosidase activity assessment • telomerase activity determination • cell cycle analysis
12	Primary neurons (Hippocampal/ cortical)	Rat/ Mouse	Dissociated neuronal cells	• axo-dendritic branching and growth • synaptic alteration • mitochondrial H_2O_2 production • cell viability • mitochondrial membrane potential • respiratory chain activity
13	Primary neurons (Hippocampal/ cortical)	Human	Neurons	• Aβ accumulation • synaptic dysfunction, • tau pathology • neurodegeneration • drug screening • neurotoxicity
14	iPSC-Derived Neurons	Human	Neurons	• $A\beta_{40}$ and $A\beta_{42}$ production • oxidative stress • RNA-seq • neuroinflammation

(miRNAs) involved in inflammatory pathways helps dissect the role of microglia in AD-related neuroinflammation. For example, transfecting BV2 cells with plasmids encoding IL-1β or TNF-α can simulate inflammatory responses seen in AD, allowing researchers to explore the effects of neuroinflammation on neuronal survival.[19]

H4 cells

Type: human neuroglioma cell line. Application: H4 cells are used in AD research to study tau protein pathology, a major component of neurofibrillary tangles in AD. Researchers often use these cells to explore the mechanisms of tau phosphorylation and aggregation, as well as the effects of potential therapeutic compounds on tau pathology.[20] Furthermore, metabolomic and proteomic investigation were performed in *APOE4*-carrying H4 neuroglioma cells to explore AD pathophysiology.[21]

C6 cell line

Type: rat astroglioma cell line. Application: Apoptosis is observed in rat astroglioma C6 cells treated with $A\beta_{25–35}$ or $A\beta_{1–42}$ where melatonin notably avoided intracellular calcium overload generated by $A\beta_{1–40}$, successfully suppressed nitric oxide generation induced by $A\beta_{1–42}$, and directly protected cells from free radical damage.[22]

3xTg-AD mouse-derived cells

Type: derived from triple-transgenic AD mouse model. Application: Cells derived from the 3xTg-AD mouse model, which carries mutations in AβPP, presenilin-1, and tau, are used to study the interplay between amyloid plaques and tau tangles. These cells are instrumental in examining the combined effects of these pathological features and testing therapeutic strategies aimed at multiple targets in AD.[23]

HEK293 cells

Type: human embryonic kidney cell line. Application: HEK293 cells (Figure 3(a)) are used in AD research primarily for their ease of genetic manipulation. They are commonly transfected with genes related to AD, such as mutant forms of AβPP, tau, presenilin-1, and presenilin-2, to study the molecular pathways involved in amyloid plaque formation and tau phosphorylation.[24–26] In vitro $A\beta_{42}$-CFP-overexpressed HEK293 cell line were utilized to study the role of Selenoprotein M in antioxidation, neuroprotection, and intracellular calcium regulation, which are the key factors for preventing the onset and progression of AD by reducing the intracellular $A\beta_{42}$ aggregation rates and by mitigating the intumescentia of mitochondria.[27]

IMR-90 and WI-38 cells

Type: human fibroblast cell lines. Application: IMR-90 and WI-38 are primary fibroblast cell lines derived from lung tissue and are commonly used to study cellular aging. These cells exhibit features of cellular senescence, a state of irreversible growth arrest that is associated with aging and has been implicated in the progression of AD. They provide a model to investigate the role of senescence in neurodegeneration and the impact of senolytic drugs.[28,29] Fibroblasts from AD patients and age-matched and young controls were studied to examine levels of oxidative stress and apoptotic markers.[30]

Rat/mouse cortical/hippocampal neurons

Type: primary human neurons derived from the hippocampus/cortex. Axonal growth, dendritic branching, and synaptic features have been studied using primary hippocampal or cortical cultured neurons in their different developmental stages (Figure 3(b)).[31,32] Moreover, cell biology features like cell viability, autophagy, apoptosis, mitochondrial biogenesis and dynamics, endoplasmic reticulum stress have also been regularly studied in primary neuronal cells. For example, primary neuronal cultures of AβPP mice (Tg2576 line) and wild-type mice were prepared and treated with MitoQ (20 nM), SS31 (1 nM), and resveratrol (5 μM), and were used for experiments to investigate mitochondrial features in AD.[11] In a similar fashion, in vitro Aβ peptide treated mouse hippocampal neurons were also used as AD model to study mitochondrial H_2O_2 production.[33] In another instance, cell viability, mitochondrial membrane potential, and the respiratory chain activity were measured in $A\beta_{25–35}$ treated rat hippocampal neurons.[34]

Human cortical neurons

Type: primary human neurons derived from the cerebral cortex. Application: Human cortical neurons are primary cells isolated from the cerebral cortex of human brains and are considered highly relevant for studying AD. These neurons closely mimic the cellular environment of the human brain, making them ideal for exploring the effects of AD-related pathology, such as amyloid-beta accumulation, tau phosphorylation, and neuroinflammation. Human cortical neurons are also used in drug screening and neurotoxicity studies, providing a more physiologically relevant model compared to immortalized cell lines.[35] In vitro $A\beta_{1–42}$ peptide treated human cortical neurons were used to study AD-induced apoptosis and effects of antioxidants on this phenomenon.[36]

Human hippocampal neurons

Type: primary human neurons derived from the hippocampus. Application: The hippocampus is a critical region of the

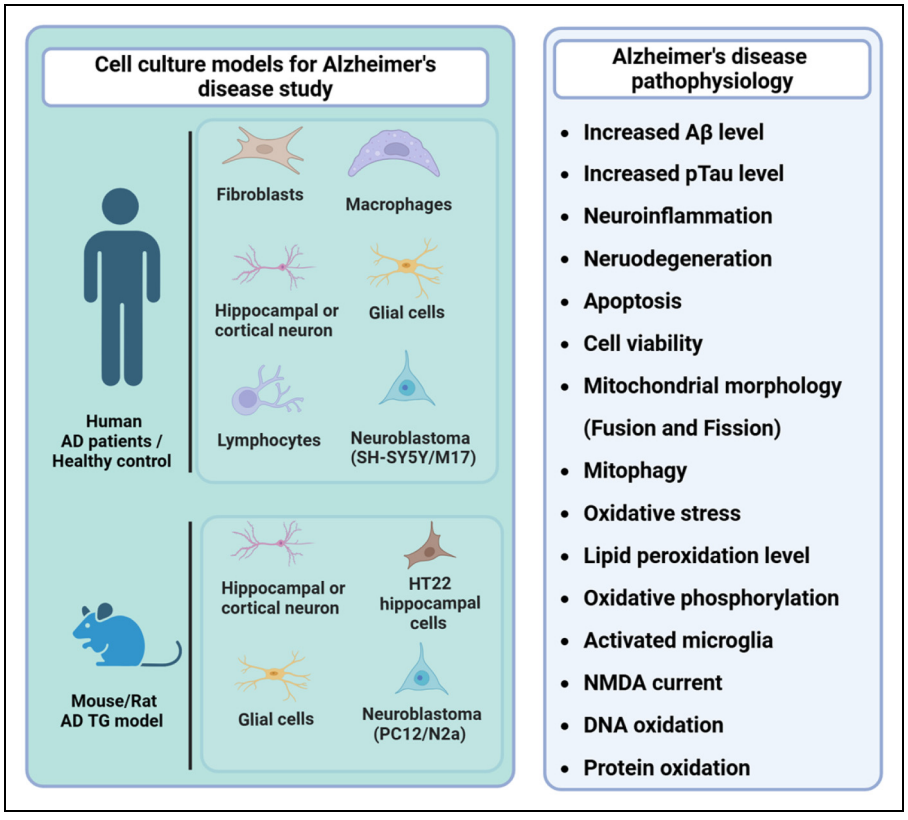

Figure 2. Different types of cell lines and their applications in AD pathophysiology study.

brain involved in memory and learning, and it is one of the first areas affected in AD. Human hippocampal neurons are used to study the mechanisms of synaptic dysfunction, tau pathology, and neurodegeneration specific to the hippocampus. These cells provide insights into the region-specific vulnerabilities in AD and are instrumental in researching therapeutic interventions targeting hippocampal dysfunction.[37,38] Transfecting human hippocampal neurons with AβPP or tau plasmids allows researchers to examine synaptic dysfunction and axonal transport defects, both key features of AD.[39]

Induced pluripotent stem cells-derived neurons

Type: induced pluripotent stem cells (iPSCs). Application: iPSCs can be reprogrammed from somatic cells of AD patients and differentiated into neurons, offering a patient-specific model to study the genetic and environmental factors contributing to AD. These models are particularly valuable for exploring the impact of genetic mutations on neuronal function and for screening drugs tailored to individual genetic profiles.[40] Transfection is commonly used in these cells to correct AβPP, PSEN1/2, or MAPT mutations, allowing for patient-specific studies of AD pathology. CRISPR-Cas9 technology is often employed for genome editing in iPSC-derived neurons to either introduce or repair AD-associated mutations, enabling direct comparisons between wild-type and mutant neurons. For example, transfecting iPSC-derived neurons with CRISPR-Cas9 components to correct mutations in AβPP or PSEN1 provides insights into the specific genetic contributions to Aβ accumulation.[40]

Implications iPSCs cell culture for Alzheimer's disease

Human iPSC technologies have evolved over the past decade and created a paradigm shift in preclinical research through its utility of developing 2D and 3D human brain organoids.[41] Researchers has the sophisticated techniques and tools now to differentiate all major brain cell types from iPSCs (Figure 4) which creates the opportunity to develop increasingly complex co-culture system emulating human brain in a dish.[42,43] Cortical neurons generated from human iPSCs in a mouse glia-supported co-culture (Figure 5) are becoming a popular model for studying patient specific disease profile. iPSC-derived cells can be used to mimic many of the cellular processes disrupted in AD in vitro, and co-culture platforms are starting to provide light on the intricate relationships that arise between different brain cell types during neurodegeneration.[44] Considering these, iPSC-based technologies have

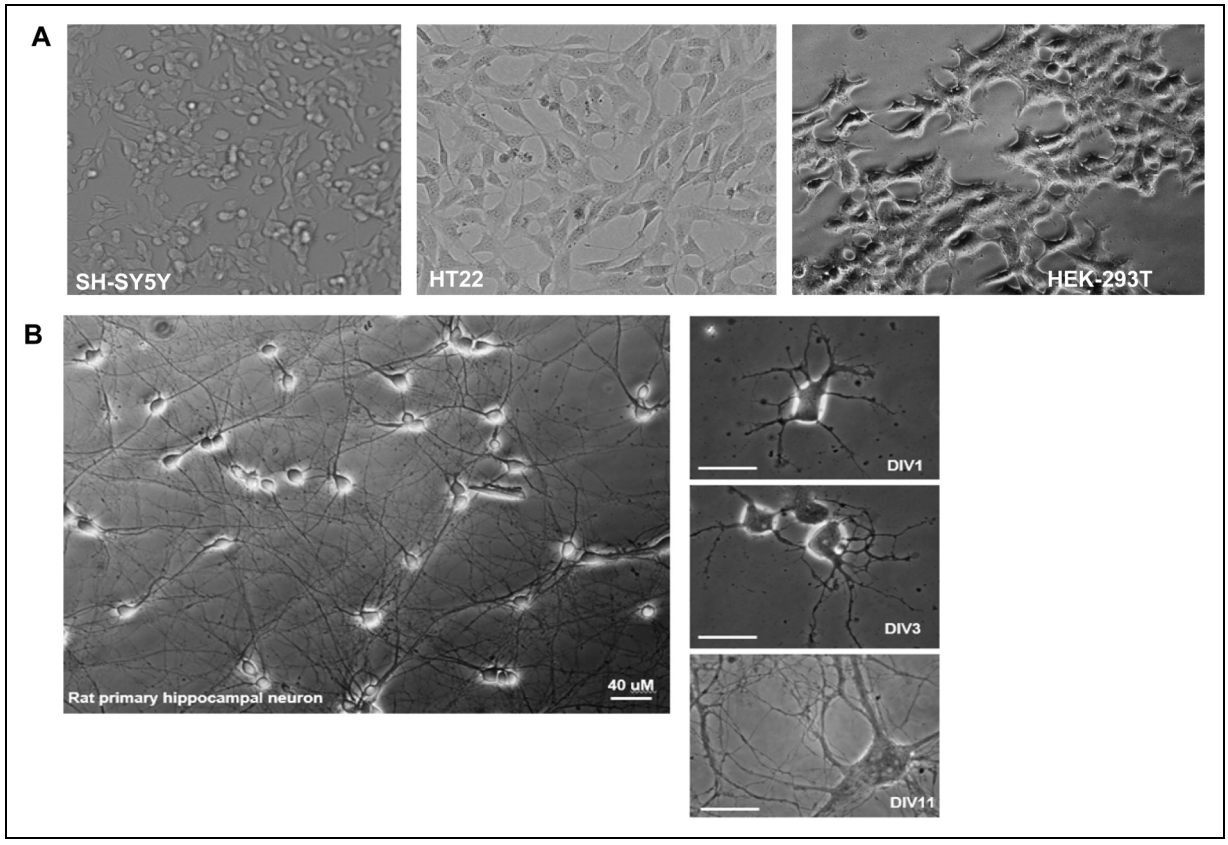

Figure 3. Example images of different cell lines (A) and rat hippocampal neurons (B). Developmental stages are shown at DIV1, DIV3, and DIV11. Scale bar, 40 μM.

great potential to further attempts to treat neurodegenerative diseases like AD.

Takahashi and Yamanaka initially demonstrated in 2006–2007 that the retroviral transfer of four transcription factors, referred to as Yamanaka's factors—Oct4, Sox2, Klf4, and c-Myc—is sufficient for cellular reprogramming of mouse or human skin fibroblasts into stem-cell-like cells known as iPSCs.[45,46] The technique of reprogramming iPSCs has advanced significantly in recent years with improved reprogramming techniques using novel delivery systems in order to ensure functional derivatives for clinical applications. Non-integrating viral and non-viral vectors, substitute reprogramming factors, small molecules, inhibitors of particular signaling or epigenetic modulators facilitate reprogramming efficiency.[47,48] These modified iPSCs have undergone differentiation into the intended subtypes of neurons, astrocytes, or microglia. Therefore, patient-specific iPSC-derived neural cells have been utilized in cell biology, genetic manipulation, disease modeling, drug discovery, biomarker study, toxicological testing, and regenerative medicine (Figure 1). The development of more widely used iPSC-based personalized medications will be made possible by future advancements in iPSC technology employing patient specific cells.

Recently, neuroscientists have many viable protocols for reprogramming human primary cells like fibroblasts (AD patients versus healthy control) into iPSCs which is then differentiated to neurons.[49–51] For example, Israel et al. (2012) collected fibroblasts from two patients with familial AD, two with sporadic AD and two non-demented control individuals. Next, iPSCs were generated from those fibroblasts and later differentiated to control and mutant neurons. Significantly increased levels of $A\beta_{1-40}$, phospho-tau, and active glycogen synthase kinase-3β (aGSK-3β) were found in the neurons of the AD patients compared to control,[52] whereas, Wang et al. (2018) generated human neurons from iPSCs that expressed apolipoprotein E4 (*APOE4*), one of major genetic risk factor for AD. They found that applying a small-molecule structural corrector to *APOE4*-expressing neurons resulted in a dose-dependent drop in $A\beta_{40}$ and $A\beta_{42}$ production/secretion as well as a significant reduction in p-tau levels.[53] Considering the fact that astrocytes are greatly involved in AD pathogenesis, one research group generated astrocytes from iPSCs derived from AD patients with PSEN1 ΔE9 mutation, as well as healthy and gene-corrected isogenic controls.[54]

Compared to control, AD astrocytes displayed elevated Aβ formation, increased oxidative stress, and impaired neuronal supporting function. In another occasion, impaired Aβ clearance and increased cholesterol content were reported in *APOE4* astrocytes generated from human iPSCs.[55] However, there are still many unanswered questions regarding the pathophysiology of AD due to lack of appropriate models that can accurately recreate the multistage intercellular interactions seen in AD brains in humans. Nowadays scientists are making their efforts to make 3D co-culture mix of neurons, astrocytes and microglia to emulate human brain like organoids in a dish to investigate AD progression and development. Park et al. (2018) utilized a three-dimensional (3D) microfluidic platform to develop a 3D human AD co-culture model incorporating iPSC-derived neurons, astrocytes, and immortalized human microglia (Figure 4).[56] This novel model recapitulated AD phenotypes like Aβ aggregation, phosphorylated tau accumulation along with microglial recruitment and neuroinflammatory activity.

Advantages and challenges in cell culture studies

Preparing and maintaining a uniform and homogenous cell culture system in vitro has both advantages and challenges. Cell culture allows us for proper control of the physicochemical environment, such as temperature, pH, and oxygen tension in our experiment which guide us to get a consistent and reproducible results. We can study the physiology and biochemistry of cells in a culture flask/dish where cells can also be exposed to chemicals or drugs directly. However, scientists should follow a well-written established protocol. Cell culture medium should be chosen carefully to ensure optimum growth of the cells. Moreover, coating material for the surface of plate/flask should also be appropriate. Serum and other growth factors like BDNF, VEGF, and FGF are being used to maintain a robust and healthy cell condition. DNA, RNA or protein samples should be collected when the cells are at the optimum growth condition. It is well known that production of proteins is at peak when the cells are at 60–70% confluency of the growth flask. For rat hippocampal primary neurons in culture, different proteins are expressed in different stage of their growth namely axonal growth (day in vitro 1–3), dendritic branching stage (day in vitro 5–9), spine development and synapse generation (day in vitro 12–18).

Researchers should cautiously choose the time of harvesting the biological samples based on their desired study. Moreover, collection methods of protein and specificity of the antibody is crucial for obtaining reliable data for western blotting and immunofluorescence study. Taken together, we should consider the selection of proper growth medium, growth surface, environment (temperature, pH, and oxygen status), and growth phase of cells to establish a homogenously grown consistent and reproducible culture system.

Methods implemented in AD cell models

After transfecting cell lines in aging and AD research, several assays can be performed to evaluate the effects of gene expression, protein production, and cellular functions (Figure 6). These assays help researchers investigate key pathological mechanisms like Aβ aggregation, tau phosphorylation, oxidative stress, inflammation, and neuronal dysfunction. Below are the common assays that can be performed.

Western blotting

Purpose: to analyze protein expression levels and post-translational modifications (e.g., phosphorylation, acetylation). Application: After transfecting cells with genes like AβPP, PSEN1/2, or MAPT (tau), western blotting can be used to detect the expression of Aβ, tau, phosphorylated tau, and other disease-related proteins.

Example: Detecting changes in the levels of amyloid-beta or phosphorylated tau in N2a or SH-SY5Y cells transfected with mutant AβPP or tau plasmids.[57]

ELISA (enzyme-linked immunosorbent assay)

Purpose: quantitative detection of proteins, peptides, or cytokines secreted into the culture media or present in cell lysates. Application: ELISA is frequently used to measure $A\beta_{40/42}$ levels in cell culture supernatants after transfecting neurons or neuroblastoma cells with AβPP or PSEN1/2 constructs.

Example: Measuring Aβ production from transfected N2a or SH-SY5Y cells to evaluate the effects of different mutations or drug treatments on amyloidogenic processing.[58]

Immunocytochemistry (ICC)/immunofluorescence (IF)

Purpose: to visualize the localization and expression of specific proteins within cells. Application: ICC or IF can be used to observe the distribution of proteins like Aβ, tau, or synaptic markers in transfected cells. These techniques can also assess cellular processes such as tau aggregation, synaptic dysfunction, or oxidative stress responses.

Example: Visualizing tau phosphorylation or Aβ aggregation in primary neurons or HT22 cells transfected with tau or AβPP constructs.[59]

Luciferase reporter assay

Purpose: to measure gene promoter activity or transcription factor binding. Application: Transfection with luciferase

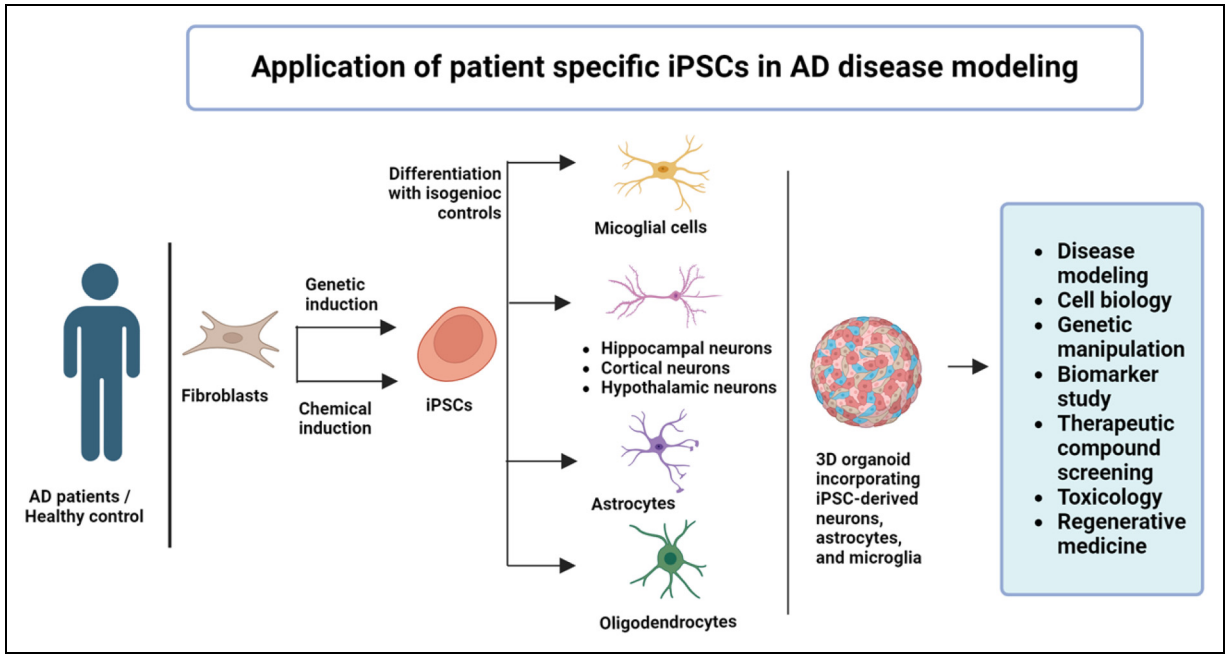

Figure 4. Schematic illustration showing AD disease modeling using human iPSCs derived brain cells.

reporter plasmids can be used to monitor the activity of AD-related pathways, such as Nrf2 (oxidative stress), NF-κB (inflammation), or CREB (synaptic plasticity).

Example: Using a luciferase reporter assay to evaluate Nrf2 pathway activation in HT22 cells after oxidative stress or transfection with Nrf2-regulated genes.[60]

RNA expression analysis (qPCR or rt-qPCR)

Purpose: to quantify gene expression at the mRNA level. Application: After transfection, qPCR can be used to analyze the expression levels of AD-relevant genes, such as AβPP, PSEN1, MAPT, or BACE1, as well as inflammatory or oxidative stress markers.

Example: Measuring changes in AβPP or BACE1 mRNA levels in SH-SY5Y cells transfected with small interfering RNA (siRNA) or CRISPR constructs targeting these genes.[61]

Mitochondrial function assays

Purpose: to assess mitochondrial health, respiration, and bioenergetic function. Application: Given the role of mitochondrial dysfunction in both aging and AD, assays like the Seahorse XF Analyzer can measure oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) in transfected cells.

Example: Analyzing mitochondrial respiration and glycolysis in N2a or HT22 cells after transfection with AβPP or tau constructs, which are linked to mitochondrial dysfunction in AD.[62]

Reactive oxygen species assay

Purpose: to measure intracellular ROS levels, indicating oxidative stress. Application: Cells transfected with AD-relevant genes like AβPP, PSEN1, or MAPT can be tested for oxidative stress, which plays a critical role in both aging and neurodegeneration.

Example: Using ROS-sensitive fluorescent dyes (e.g., DCFDA) to measure oxidative stress in transfected HT22 cells, which are prone to glutamate-induced oxidative damage.[15]

Apoptosis assays

Purpose: to detect and quantify cell death via apoptotic pathways. Application: In AD research, apoptosis is a significant factor in neuronal loss. Transfected cells can be assessed for apoptotic markers like caspase activation, DNA fragmentation (TUNEL assay), or annexin V staining.

Example: Measuring apoptosis in SH-SY5Y cells transfected with mutant tau or AβPP, both of which contribute to neurodegeneration in AD.[63,64]

Synaptic function assays

Purpose: to measure synaptic activity, plasticity, and integrity. Application: Synaptic dysfunction is a hallmark of AD. Transfected cells can be tested for synaptic markers (e.g., synaptophysin, PSD95) or synaptic transmission using electrophysiological methods.

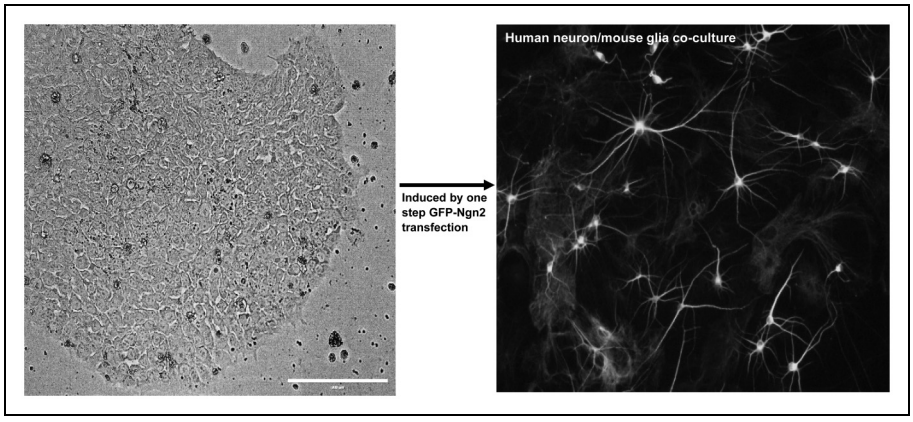

Figure 5. Induction of human cortical neurons from patient's IPSCs. Human iPSCs were transfected with GFP-Ngn2 plasmid to differentiate into cortical neurons in a mouse glia supported environment. Scale bar: 200 μm.

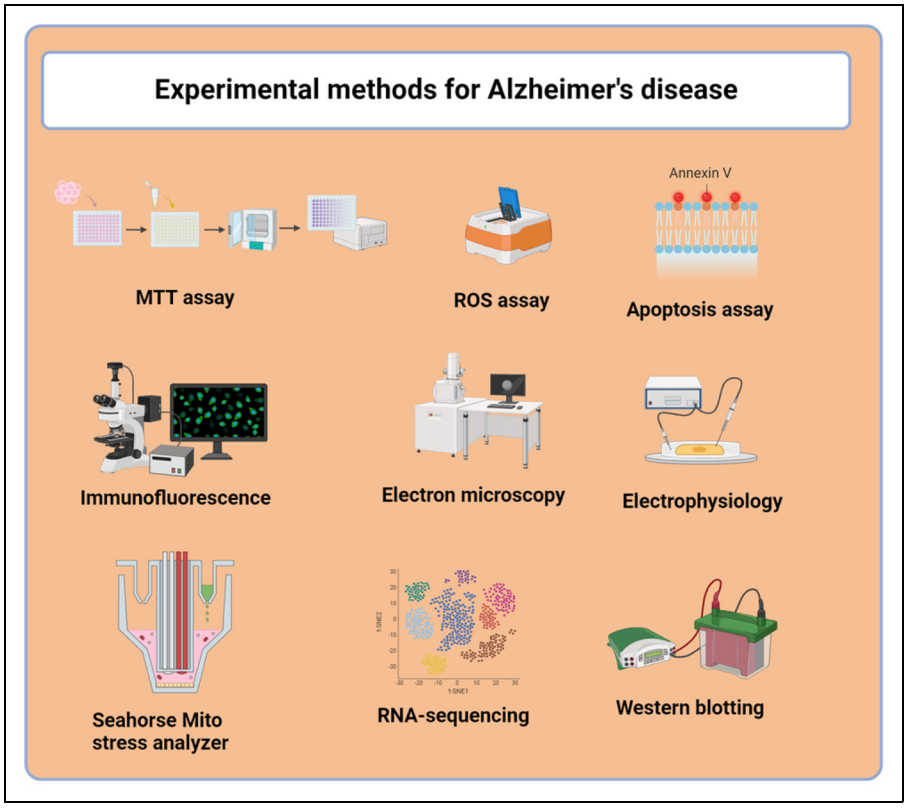

Figure 6. Methods applied in AD cell culture model to investigate AD pathophysiology. For getting a statistically significant difference in values between experiment group versus control, number of biological replicates, n = 4.

Example: Detecting synaptic deficits in human cortical neurons or SH-SY5Y cells transfected with AβPP or tau constructs using electrophysiological recordings or synaptic marker quantification.[65]

Microglial activation assay

Purpose: to study neuroinflammation by analyzing the activation of microglia. Application: Transfecting microglial cells (e.g., BV2 cells) with AD-relevant genes can help assess the role of inflammation in disease progression. After transfection, assays can measure the release of inflammatory cytokines like IL-1β, TNF-α, and IL-6 using ELISA or multiplex cytokine assays.

Example: Measuring cytokine release from BV2 cells after transfection with mutant AβPP or tau to model microglial activation in AD.[66]

Autophagy assays

Purpose: to measure the activity of autophagy, a key pathway involved in protein degradation and cellular homeostasis. Application: Since dysregulation of autophagy is implicated in AD, transfected cells can be tested for markers of autophagy, such as LC3 conversion and p62 degradation, using western blot or immunofluorescence.

Example: Assessing autophagy in N2a cells transfected with mutant AβPP or PSEN1 constructs, as impaired autophagy contributes to the accumulation of misfolded proteins in AD.[67]

Live cell imaging

Purpose: to monitor dynamic cellular processes in real-time. Application: Live-cell imaging can be used to study protein aggregation (e.g., Aβ or tau), mitochondrial dynamics, calcium homeostasis, or synaptic activity in transfected cells.

Example: Using fluorescence-tagged tau to observe their aggregation and trafficking in primary neurons or iPSC-derived neurons.[68]

A wide range of assays can be performed on transfected cell lines to investigate various aspects of aging and AD pathology, including protein expression, oxidative stress, mitochondrial function, inflammation, apoptosis, and synaptic dysfunction. These assays provide critical insights into the molecular and cellular mechanisms underlying neurodegeneration and help evaluate the efficacy of potential therapeutic interventions.

Transfection as a method to create AD cell culture model

Transfection is a method used in molecular biology to introduce nucleic acids (such as DNA or RNA) into eukaryotic cells. This technique allows researchers to study gene function and regulation, protein expression, and the effects of genetic mutations, as well as to create genetically modified cell lines for various applications. Transfection can involve plasmids, siRNAs, miRNAs, or CRISPR components to modulate gene expression or genome editing.

Transfection is a critical tool in molecular biology, enabling the functional analysis of genes and the development of genetically modified cells for research. With advancements in transfection reagents and methods, scientists continue to improve the efficiency, specificity, and applicability of this technique across a broad range of biological fields.

Transfection is a valuable technique in aging and AD research, especially for investigating gene function, protein expression, and cellular processes involved in neurodegeneration. In cell lines used for studying aging and AD, transfection allows researchers to modulate the expression of genes associated with key pathological features, such as amyloid-beta production, tau phosphorylation, oxidative stress, and inflammation.

Types of transfections

Transient transfection. In transient transfection, the introduced nucleic acids do not integrate into the host genome and are only expressed temporarily. This method is useful for short-term studies, such as gene expression analysis, reporter assays, or drug screening.

Applications: Protein expression, RNA interference, functional assays

Advantages: Rapid expression, easier protocols

Disadvantages: Temporary gene expression, loss of expression after a few days

Stable transfection. In stable transfection, the introduced DNA integrates into the host cell genome, leading to long-term and stable expression of the gene. This requires the selection of transfected cells using markers like antibiotic resistance.

Applications: Long-term studies, generation of cell lines, continuous protein production

Advantages: Permanent gene expression, useful for long-term experiments

Disadvantages: Time-consuming, requires selection processes

Methods of transfection

Chemical transfection. Lipofection (Liposome-based): Lipid-based transfection reagents encapsulate nucleic acids and facilitate their entry into cells by fusing with the cell membrane. Lipofection is efficient for both transient and stable transfection.

Calcium phosphate precipitation: This method forms a precipitate containing the DNA, which is taken up by cells via endocytosis.

Polyethylenimine (PEI): A polymer that forms complexes with DNA, allowing its uptake by the cell.

Physical transfection. Electroporation: High-voltage electrical pulses create pores in the cell membrane, through which nucleic acids enter the cell. It is highly efficient but can cause cell damage.

Microinjection: Direct injection of nucleic acids into the nucleus or cytoplasm of individual cells using a fine needle. This method is precise but labor-intensive and is used for specialized applications like single-cell studies.

Biolistic (Gene Gun): Nucleic acids are attached to micro-particles (typically gold or tungsten) and are physically shot into cells.

Viral transfection (transduction). In viral transfection, viruses such as lentivirus, adenovirus, or retrovirus are engineered to carry the gene of interest. The virus infects the host cell, delivering the genetic material.

Advantages: High efficiency, especially in hard-to-transfect cells

Disadvantages: Risk of insertional mutagenesis, labor-intensive, biosafety considerations

Applications of transfection

Gene function studies: Transfection is used to overexpress or knock down genes, helping researchers study their function in various biological processes.

Protein production: Transfection allows for the expression of recombinant proteins in cell lines for research or therapeutic purposes.

Gene editing: Techniques like CRISPR-Cas9 rely on transfection to introduce components needed for genome editing into cells.

Drug discovery and screening: Cells transfected with disease-relevant genes are used to screen potential drug candidates.

Reporter gene assays: Transfection of reporter constructs, such as luciferase or GFP, helps monitor gene expression and promoter activity.

Challenges and considerations in transfecting cells in culture

Cell Type: Some cell types, such as primary neurons or immune cells, are more difficult to transfect, and specific optimization is required.

Efficiency: The efficiency of transfection can vary depending on the cell line, nucleic acid type, and method used.

Cytotoxicity: Some transfection reagents and methods can induce cytotoxic effects, necessitating careful optimization to minimize cell death.

Considering the above-mentioned factors, cell type specific transfection methods are listed below to optimize the validity and reproducibility of the experiment findings:

Lipofection: Commonly used for SH-SY5Y and N2a cells to introduce plasmids or siRNAs.

Electroporation: Often used for primary neurons and iPSC-derived neurons, as these cells are more difficult to transfect with chemical methods.

Viral transfection (transduction): Lentivirus or adenovirus-mediated transfection is frequently used in primary neurons or difficult-to-transfect cells, providing high efficiency and stable expression.

Transfection is essential in aging and AD research for modeling disease mechanisms, studying gene function, and testing potential therapies. By using various cell lines such as N2a, SH-SY5Y, HT22, and primary neurons, researchers can explore the molecular underpinnings of AD and age-related neurodegeneration. Advances in transfection technologies and gene-editing tools like CRISPR-Cas9 are making it increasingly possible to create more accurate models of the disease, leading to better therapeutic insights.

Concerns regarding statistical significance across experimental transfections

Some reviewers have expressed concerns about the similarity in p-values across different experimental transfections, particularly when using the same cell line, such as HT22 cells transfected with mutant AβPP and mutant tau plasmids. It is essential to address this scientifically and reinforce that this outcome is both expected and justified, given the experimental design.

Biological consistency of cell lines

When employing the same cell line for different experimental transfections, the cellular background remains identical. HT22 cells, for instance, exhibit the same baseline physiological characteristics across transfections. This intrinsic uniformity naturally leads to comparable biological responses when subjected to different plasmid conditions (mutant AβPP and mutant tau). Since the foundational cellular properties do not change, statistical similarities in p-values are not just expected, but are a reflection of consistent experimental conditions. The differences observed are more likely due to the specific plasmid-induced effects, rather than cellular variability.

Controlled experimental environment

In highly controlled experiments, factors such as cell culture conditions, transfection efficiency, and assay timings are meticulously standardized. These controlled parameters further minimize experimental variation, contributing to similar P-values. The transfection of mutant AβPP or tau into the same cell line under uniform conditions allows for a more accurate comparison of how each plasmid impacts cellular behavior. Consequently, any variations in cellular response are due to the specific genetic constructs and not the experimental environment, reinforcing the consistency of statistical outcomes.

Use of a common control group

The decision to use a shared control group across different transfection conditions strengthens the robustness of the data. This approach allows researchers to streamline comparisons between different conditions while eliminating unnecessary experimental variability. By maintaining an identical control across plasmid transfections, it becomes evident that any observed effects are attributable to the differences in transfection conditions rather than changes in control behavior. This enhances the interpretability of the data, as it directly compares how the same cell line responds to different plasmids relative to a consistent baseline.

Efficient resource utilization

In high-cost, resource-intensive experiments—such as those involving transmission electron microscopy (TEM), cell survival assay, seahorse bioanalyzer, and immunofluorescence microscopy—the use of a common control group is not only scientifically sound but also economically strategic. TEM, known for its high cost and time requirements, benefits significantly from shared controls, reducing the number of samples required without compromising data integrity. By minimizing redundancy in control samples, this approach enables more efficient experimentation, ensuring that results remain statistically robust while economizing on limited resources.

Scientific rationale for similar p-values

The uniformity in p-values across these transfection experiments is not a sign of experimental weakness but rather an indicator of methodological rigor. By using the same cell line under similar conditions, the baseline cellular properties remain constant, and therefore, the variance between experimental groups remains minimal. This consistency is critical in high-throughput studies, where the goal is to isolate the effects of specific interventions (in this case, mutant AβPP and tau) without introducing noise from experimental inconsistencies.

The similarity in p-values reflects the controlled, uniform conditions under which the experiments were performed, as well as the consistency in the cellular model used. By maintaining a shared control group and adhering to strict experimental protocols, the results are both reliable and scientifically valid. This approach not only minimizes variability but also ensures the robustness of the data, providing a clear understanding of how different plasmid transfections affect the same cellular model. Thus, the statistical outcomes are justified, logical, and indicative of the study's rigorous design (Figure 7).

Discussion

Transfection of cell lines in aging and AD research plays a crucial role in uncovering the molecular mechanisms of neurodegeneration and the potential therapeutic targets for treating or slowing the progression of these diseases. By introducing genetic constructs into cells such as AβPP and PSEN1 (which are linked to Aβ pathology) or MAPT (related to tau aggregation), researchers can mimic key features of AD and observe the effects on

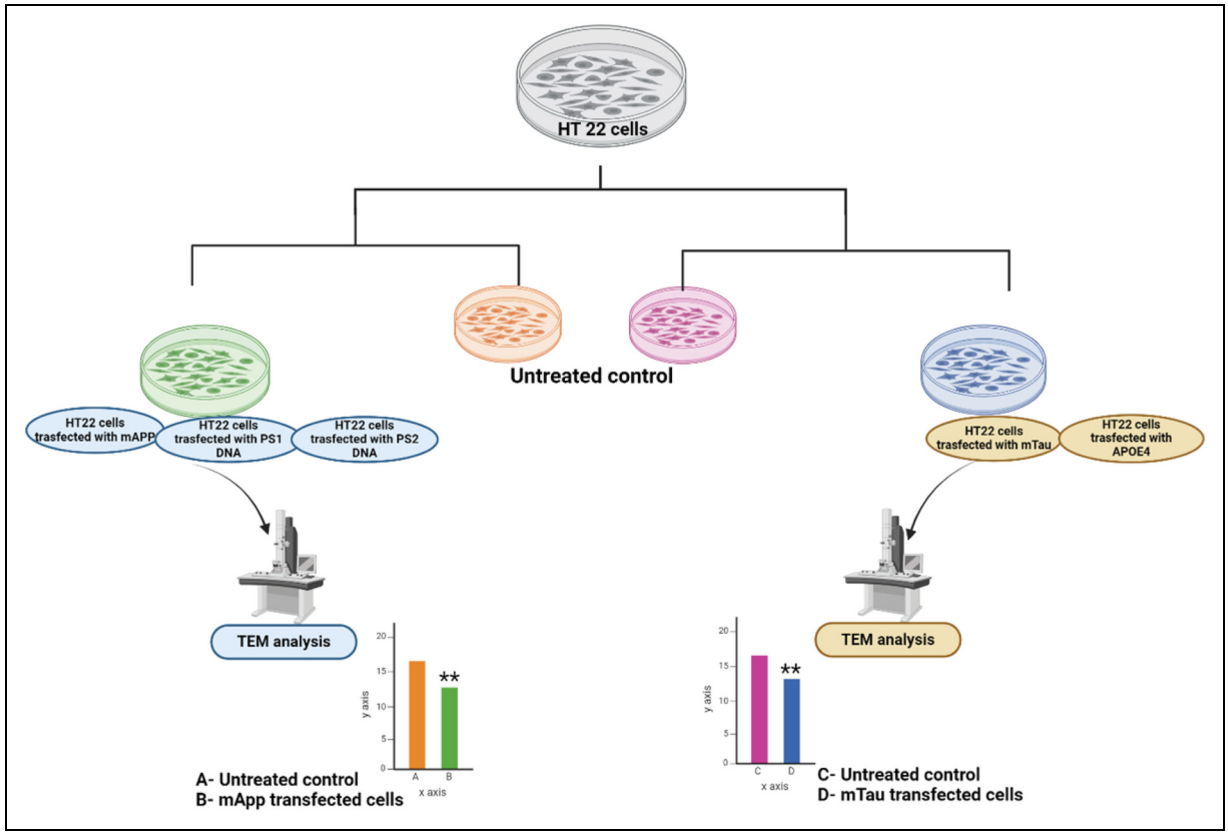

Figure 7. The same cell line is used under different transfection conditions; the baseline cellular properties remain consistent. The similarity in p-values can be attributed to the uniform nature of the cells, which respond similarly under controlled conditions when subjected to different transfection agents.

various cellular processes. After successful transfection, multiple assays are typically conducted to examine the impacts of these genetic modifications on Aβ production, tau hyperphosphorylation, oxidative stress, synaptic dysfunction, inflammation, and neuronal death. One of the main advantages of using transfected cell lines, such as N2a, SH-SY5Y, and HT22, is the ability to manipulate specific genes and pathways involved in aging and neurodegeneration, allowing for precise control and investigation of disease-related processes. For example, overexpressing mutant AβPP in N2a cells can increase Aβ production, enabling researchers to assess the effects of various substances, drugs, or genetic interventions on amyloidogenic pathways. Similarly, transfection of SH-SY5Y or primary human neurons with tau constructs can be used to study the mechanisms behind tau aggregation, a key hallmark of AD.[69–71]

Use/reuse of common controls in experiments

An essential consideration in designing transfection experiments is the need for appropriate controls. In experiments where different substances or treatments are being tested on transfected cells, the use of the same control groups is not only valid but also highly beneficial, provided that all experiments are performed under identical conditions at the same time. This approach minimizes variability and ensures that any observed differences in outcomes can be attributed directly to the substances or treatments being tested, rather than to variations in experimental conditions. For example, if HT22 cells are transfected with a mutant AβPP and tau-expressing plasmid and are treated with different therapeutic compounds to test their effects on AβPP and tau phosphorylation, the control group (transfected but untreated cells) can serve as a baseline across all treatments. This allows for direct comparisons of how each compound affects AβPP and tau phosphorylation relative to the untreated control. Using a single control group for multiple experimental conditions in the same batch of transfected cells ensures consistency and reduces the number of experimental replicates required, saving both time and resources (Figure 8).

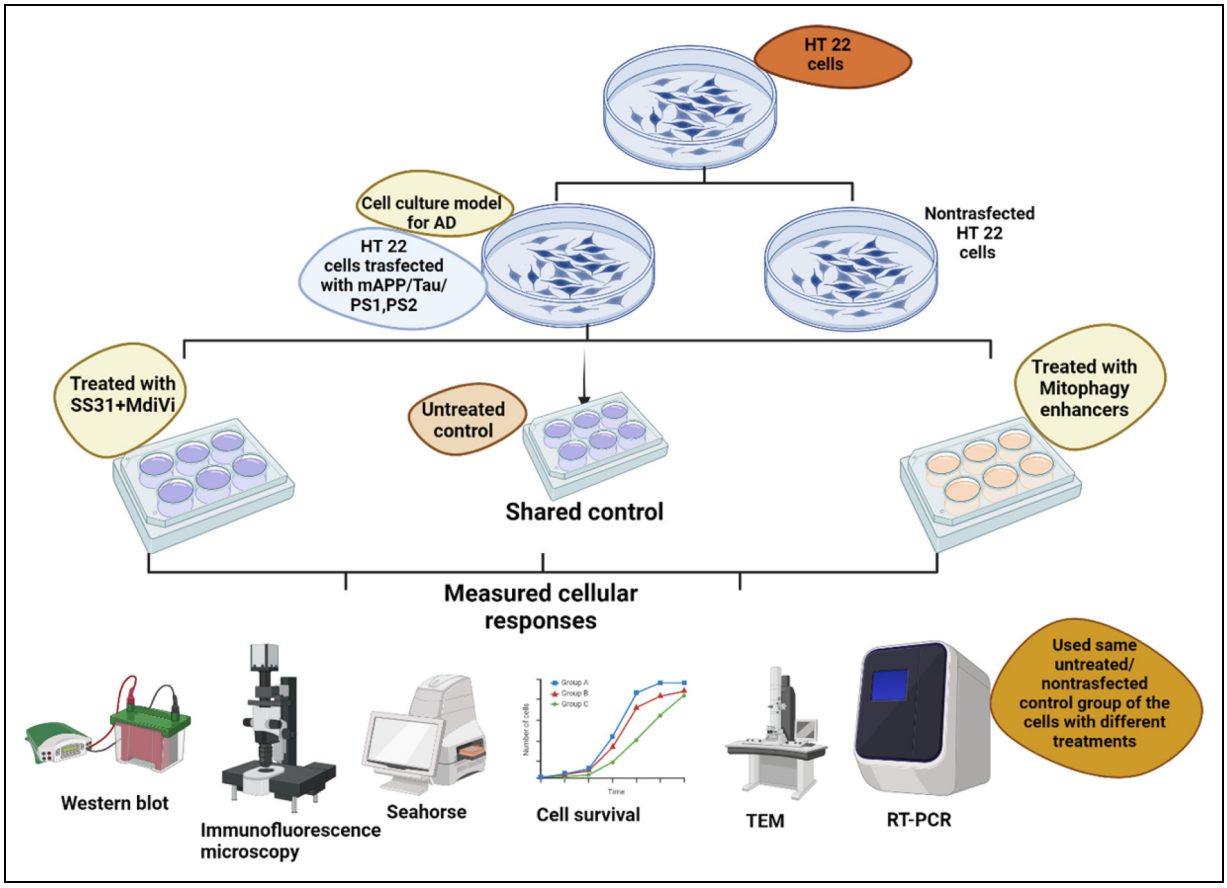

Figure 8. Using the same untreated control group offers the advantage of economizing on resources, particularly for expensive assays such as TEM (transmission electron microscopy), seahorse bioanalyzer, and cell survival assay. By sharing control across conditions, we can reduce the number of control samples, minimizing the financial and logistical burden of complex and costly techniques.

Table 2. Application of different types of cell line for Alzheimer's disease studies in Reddy lab.

Sl no.	Cell line	Experiments	Major findings	Reference
1	N2a cells expressing either mutant human AβPP or WT human AβPP	Immunoblotting	Aβ is localized to the inner mitochondrial membrane	[72]
2	N2a cells treated with MitoQ, SS31, and resveratrol, and then incubated with Aβ	Electron microscopy, gene expression analysis, and biochemical methods.	Mitochondria-Targeted Antioxidants Protect Against Aβ Toxicity	[11]
2	primary neurons from AβPP transgenic mice	Immunofluorescence, immunoblotting and ELISA	over-accumulation of oligomeric Aβ, impaired mitochondrial biogenesis and dynamics, and synaptic degeneration	[73,74]
3	HT22 cells transfected with mutant AβPP cDNA construct	Mitochondrial functional assays by measuring H_2O_2, lipid peroxidation, cytochrome c oxidase activity, and MTT determination, and apoptosis assay, mRNA expression Assay, immunoblotting	Abnormal mitochondrial dynamics and defective biogenesis, reduced mitophagy, & reduced dendritic spines	[75]
4	SH-SY5Y cells incubated with Aβ peptide 1–42	Quantification of mitochondrial dynamics, biogenesis and synaptic genes expression using real-time RT-PCR	Aqua-soluble DDQ protects AD neurons from Aβ-induced mitochondrial and synaptic toxicities	[76]
5	Human skin fibroblast and B-lymphocytes cell culture	Real-time RT-PCR	miR-455-3p level was observed in the cells of sporadic AD patients compared to healthy controls	[77,78]
6	HT 22 cells transfected with mutant Tau cDNA construct	Immunoblotting, Cell survival assay, Mitochondrial functional assay, qRT-PCR, TEM, Immunostaining	Showed protective effects of mitophagy enhancers against phosphorylated tau-induced mitochondrial and synaptic toxicities in AD	[16]

Assay considerations

After transfection, various assays can be performed to evaluate cellular responses to aging and AD-related stressors. Western blotting, for instance, allows for the detection of changes in protein levels, such as the amount of phosphorylated tau or Aβ in different treatment groups. The use of the same control for multiple experimental treatments ensures that the data can be directly compared across groups, facilitating a more accurate assessment of treatment efficacy. Similarly, in ELISA assays, where the levels of secreted Aβ peptides ($A\beta_{40}$ and $A\beta_{42}$) are quantified, using the same control ensures that the baseline Aβ secretion can be consistently measured. This is especially important when evaluating the effects of different drugs or gene knockdowns on amyloidogenic pathways. In mitochondrial respiration assays or ROS assays, using a shared control group allows researchers to determine how various treatments influence mitochondrial function or oxidative stress relative to a baseline condition. Given the critical role of oxidative stress in both aging and AD, consistency in controls helps clarify the effects of interventions on cellular health and neurodegeneration (Table 2: Reddy group articles).

Significance of control group consistency

The use of a single control group for transfection experiments performed at the same time is scientifically sound as long as the cells are handled consistently and treated identically, apart from the substance or treatment being applied. This practice is especially important in aging and AD research, where small differences in experimental conditions can introduce significant variability. Maintaining a common control group across all experimental conditions not only streamlines the research process but also enhances the robustness of the data. In AD research, subtle differences in transgene expression, oxidative stress levels, or inflammation can significantly impact the results of the study. Thus, ensuring that a single control group is used for multiple experimental treatments guarantees that differences between groups are due to the specific interventions being tested rather than confounding factors such as changes in cell culture conditions or transfection efficiency. Furthermore, this approach allows for direct comparisons between substances that may target different aspects of AD pathology. For example, one substance may primarily reduce Aβ production, while another may affect tau phosphorylation. By using the same control group, researchers can determine which substance has a more profound

effect on the disease-related processes being studied, providing a more comprehensive understanding of therapeutic potential.

Transfection of cell lines is a powerful tool in aging and AD research, allowing for the detailed study of disease-related mechanisms such as amyloid-beta generation, tau hyperphosphorylation, oxidative stress, and synaptic dysfunction. The assays performed after transfection, including western blotting, ELISA, mitochondrial respiration, and ROS analysis, provide critical insights into the cellular and molecular changes that occur in response to genetic manipulation. Using the same control group for multiple experimental treatments within the same transfection experiment ensures consistency, reduces variability, and facilitates meaningful comparisons across treatment groups. This strategy is especially important in the context of aging and AD research, where small variations in experimental conditions can lead to significant differences in outcomes. By applying a common control, researchers can more effectively assess the impact of different therapeutic substances and interventions on the cellular processes involved in neurodegeneration, paving the way for the development of more effective treatments for AD.

Acknowledgments

The authors have no acknowledgments to report.

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Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by NIH grant AG082961 (to PHR).

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Pampaloni F, Reynaud EG and Stelzer EHK. The third dimension bridges the gap between cell culture and live tissue. *Nat Rev Mol Cell Biol* 2007; 8: 839–845.
2. Huh D, Hamilton GA and Ingber DE. From 3D cell culture to organs-on-chips. *Trends Cell Biol* 2011; 21: 745–754.
3. Baker M. Reproducibility crisis: blame it on the antibodies. *Nature* 2015; 521: 274–276.
4. Xicoy H, Wieringa B and Martens GJ. The SH-SY5Y cell line in Parkinson's disease research: a systematic review. *Mol Neurodegener* 2017; 12: 10.
5. Marinelli L, Fornasari E, Di Stefano A, et al. (R)-α-Lipoyl-Gly-l-Pro-l-Glu dimethyl ester as dual acting agent for the treatment of Alzheimer's disease. *Neuropeptides* 2017; 66: 52–58.
6. Rhein V, Giese M, Baysang G, et al. Ginkgo biloba extract ameliorates oxidative phosphorylation performance and rescues aβ-induced failure. *PloS One* 2010; 5: e12359.
7. Kaur N, Kaur S, Saini M, et al. Phytochemical ginkgolide B attenuates amyloid-β1–42 induced oxidative damage and altered cellular responses in human neuroblastoma SH-SY5Y cells. *Neurodegeneration* 2020; 2: 5.
8. Bell M and Zempel H. SH-SY5Y-derived neurons: a human neuronal model system for investigating tau sorting and neuronal subtype-specific TAU vulnerability. *Rev Neurosci* 2022; 33: 1–15.
9. Sadli N, Barrow CJ, McGee S, et al. Effect of DHA and coenzymeQ10 against Aβ-and zinc-induced mitochondrial dysfunction in human neuronal cells. *Cell Physiol Biochem* 2013; 32: 243–252.
10. Sun J, Yuan B, Wu Y, et al. Sodium butyrate protects N2a cells against Aβ toxicity in vitro. *Mediators Inflamm* 2020; 2020: 7605160.
11. Manczak M, Mao P, Calkins MJ, et al. Mitochondria-targeted antioxidants protect against amyloid-β toxicity in Alzheimer's disease neurons. *J Alzheimers Dis* 2010; 20: S609–S631.
12. Eckert A, Keil U, Kressmann S, et al. Effects of EGb 761 Ginkgo biloba extract on mitochondrial function and oxidative stress. *Pharmacopsychiatry* 2003; 36(Suppl 1): S15–S23.
13. Eckert A, Keil U, Scherping I, et al. Stabilization of mitochondrial membrane potential and improvement of neuronal energy metabolism by Ginkgo biloba extract EGb 761. *Ann N Y Acad Sci* 2005; 1056: 474–485.
14. Jang JH and Surh YJ. Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death. *Free Radic Biol Med* 2003; 34: 1100–1110.
15. Li Y, Maher P and Schubert D. Requirement for cGMP in nerve cell death caused by glutathione depletion. *J Cell Biol* 1997; 139: 1317–1324.
16. Kshirsagar S, Sawant N, Morton H, et al. Protective effects of mitophagy enhancers against amyloid beta-induced mitochondrial and synaptic toxicities in Alzheimer disease. *Hum Mol Genet* 2022; 31: 423–439.

17. Fukui M and Zhu BT. Mitochondrial superoxide dismutase SOD2, but not cytosolic SOD1, plays a critical role in protection against glutamate-induced oxidative stress and cell death in HT22 neuronal cells. *Free Radic Biol Med* 2010; 48: 821–830.
18. Henn A, Lund S, Hedtjärn M, et al. The suitability of BV2 cells as alternative model system for primary microglia cultures or for animal experiments examining brain inflammation. *Altex* 2009; 26: 83–94.
19. He BP, Wen W and Strong MJ. Activated microglia (BV-2) facilitation of TNF-alpha-mediated motor neuron death in vitro. *J Neuroimmunol* 2002; 128: 31–38.
20. DeTure M, Ko LW, Easson C, et al. Tau assembly in inducible transfectants expressing wild-type or FTDP-17 tau. *Am J Pathol* 2002; 161: 1711–1722.
21. Lu L, Kotowska AM, Kern S, et al. Metabolomic and proteomic analysis of ApoE4-carrying H4 neuroglioma cells in Alzheimer's disease using OrbiSIMS and LC-MS/MS. *Anal Chem* 2024; 96: 11760–11770.
22. Feng Z and Zhang JT. Protective effect of melatonin on beta-amyloid-induced apoptosis in rat astroglioma C6 cells and its mechanism. *Free Radic Biol Med* 2004; 37: 1790–1801.
23. Oddo S, Caccamo A, Shepherd JD, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 2003; 39: 409–421.
24. Eteläinen TS, Silva MC, Uhari-Väänänen JK, et al. A prolyl oligopeptidase inhibitor reduces tau pathology in cellular models and in mice with tauopathy. *Sci Transl Med* 2023; 15: eabq2915.
25. Shi J, Ku X, Zou X, et al. Comprehensive analysis of O-glycosylation of amyloid precursor protein (APP) using targeted and multi-fragmentation MS strategy. *Biochim Biophys Acta Gen Subj* 2021; 1865: 129954.
26. Van Gassen G, De Jonghe C, Nishimura M, et al. Evidence that the beta-catenin nuclear translocation assay allows for measuring presenilin 1 dysfunction. *Mol Med* 2000; 6: 570–580.
27. Chen P, Wang RR, Ma XJ, et al. Different forms of selenoprotein M differentially affect aβ aggregation and ROS generation. *Int J Mol Sci* 2013; 14: 4385–4399.
28. Pu X, Yu S, Fan W, et al. Guiqi polysaccharide protects the normal human fetal lung fibroblast WI-38 cells from H2O2-induced premature senescence. *Int J Clin Exp Pathol* 2015; 8: 4398–4407.
29. Sikora E, Bielak-Zmijewska A, Dudkowska M, et al. Cellular senescence in brain aging. *Front Aging Neurosci* 2021; 13: 646924.
30. Moreira PI, Harris PL, Zhu X, et al. Lipoic acid and N-acetyl cysteine decrease mitochondrial-related oxidative stress in Alzheimer disease patient fibroblasts. *J Alzheimers Dis* 2007; 12: 195–206.
31. Islam MA, Sharif SR, Lee H, et al. N-Acetyl-D-glucosamine kinase promotes the axonal growth of developing neurons. *Mol Cells* 2015; 38: 876–885.
32. Islam MA, Sharif SR, Lee H, et al. N-acetyl-D-glucosamine kinase interacts with dynein light-chain roadblock type 1 at Golgi outposts in neuronal dendritic branch points. *Exp Mol Med* 2015; 47: e177.
33. Lobos P, Bruna B, Cordova A, et al. Astaxanthin protects primary hippocampal neurons against noxious effects of Aβ-oligomers. *Neural Plast* 2016; 2016: 3456783.
34. Dong W, Huang F, Fan W, et al. Differential effects of melatonin on amyloid-beta peptide 25–35-induced mitochondrial dysfunction in hippocampal neurons at different stages of culture. *J Pineal Res* 2010; 48: 117–125.
35. D'Aiuto L, Zhi Y, Kumar Das D, et al. Large-scale generation of human iPSC-derived neural stem cells/early neural progenitor cells and their neuronal differentiation. *Organogenesis* 2014; 10: 365–377.
36. Medina S, Martínez M and Hernanz A. Antioxidants inhibit the human cortical neuron apoptosis induced by hydrogen peroxide, tumor necrosis factor alpha, dopamine and beta-amyloid peptide 1–42. *Free Radic Res* 2002; 36: 1179–1184.
37. Small SA, Schobel SA, Buxton RB, et al. A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci* 2011; 12: 585–601.
38. Du AT, Schuff N, Kramer JH, et al. Different regional patterns of cortical thinning in Alzheimer's disease and frontotemporal dementia. *Brain* 2007; 130: 1159–1166.
39. D'Avanzo C, Aronson J, Kim YH, et al. Alzheimer's in 3D culture: challenges and perspectives. *Bioessays* 2015; 37: 1139–1148.
40. Yagi T, Ito D, Okada Y, et al. Modeling familial Alzheimer's disease with induced pluripotent stem cells. *Hum Mol Genet* 2011; 20: 4530–4539.
41. Sloan SA, Andersen J, Paşca AM, et al. Generation and assembly of human brain region-specific three-dimensional cultures. *Nat Protoc* 2018; 13: 2062–2085.
42. Lim CS, Kim MJ, Choi JE, et al. Dysfunction of NMDA receptors in neuronal models of an autism spectrum disorder patient with a DSCAM mutation and in Dscam-knockout mice. *Mol Psychiatry* 2021; 26: 7538–7549.
43. Goodwin SJ. A brain in a dish. *Postdoc J* 2015; 3: 31–35.
44. Penney J, Ralvenius WT and Tsai L-H. Modeling Alzheimer's disease with iPSC-derived brain cells. *Mol Psychiatry* 2020; 25: 148–167.
45. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861–872.
46. Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; 126: 663–676.
47. Singh VK, Kalsan M, Kumar N, et al. Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. *Front Cell Dev Biol* 2015; 3: 2.
48. Li J, Song W, Pan G, et al. Advances in understanding the cell types and approaches used for generating induced pluripotent stem cells. *J Hematol Oncol* 2014; 7: 50.

49. Begum AN, Guoynes C, Cho J, et al. Rapid generation of sub-type, region-specific neurons and neural networks from human pluripotent stem cell-derived neurospheres. *Stem Cell Res* 2015; 15: 731–741.
50. Ho S-M, Hartley BJ, Tcw J, et al. Rapid Ngn2-induction of excitatory neurons from hiPSC-derived neural progenitor cells. *Methods* 2016; 101: 113–124.
51. Zhang Y, Pak C, Han Y, et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron* 2013; 78: 785–798.
52. Israel MA, Yuan SH, Bardy C, et al. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* 2012; 482: 216–220.
53. Wang C, Najm R, Xu Q, et al. Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. *Nat Med* 2018; 24: 647–657.
54. Oksanen M, Petersen AJ, Naumenko N, et al. PSEN1 Mutant iPSC-derived model reveals severe astrocyte pathology in Alzheimer's disease. *Stem Cell Rep* 2017; 9: 1885–1897.
55. Lin YT, Seo J, Gao F, et al. APOE4 Causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron* 2018; 98: 1141–1154. e1147.
56. Park J, Wetzel I, Marriott I, et al. A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. *Nat Neurosci* 2018; 21: 941–951.
57. Sato N and Morishita R. The roles of lipid and glucose metabolism in modulation of β-amyloid, tau, and neurodegeneration in the pathogenesis of Alzheimer disease. *Front Aging Neurosci* 2015; 7: 199.
58. Gonçalves PB, Sodero ACR and Cordeiro Y. Natural products targeting amyloid-β oligomer neurotoxicity in Alzheimer's disease. *Eur J Med Chem* 2024; 276: 116684.
59. Rohn TT, Rissman RA, Davis MC, et al. Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. *Neurobiol Dis* 2002; 11: 341–354.
60. Suh JH, Shenvi SV, Dixon BM, et al. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proc Natl Acad Sci U S A* 2004; 101: 3381–3386.
61. Fu WY and Ip NY. The role of genetic risk factors of Alzheimer's disease in synaptic dysfunction. *Semin Cell Dev Biol* 2023; 139: 3–12.
62. Foret MK, Orciani C, Welikovitch LA, et al. Early oxidative stress and DNA damage in Aβ-burdened hippocampal neurons in an Alzheimer's-like transgenic rat model. *Commun Biol* 2024; 7: 861.
63. Song Z, He C, Yu W, et al. Baicalin attenuated Aβ (1–42)-induced apoptosis in SH-SY5Y cells by inhibiting the Ras-ERK signaling pathway. *Biomed Res Int* 2022; 2022: 9491755.
64. Löffler T, Flunkert S, Taub N, et al. Stable mutated tau441 transfected SH-SY5Y cells as screening tool for Alzheimer's disease drug candidates. *J Mol Neurosci* 2012; 47: 192–203.
65. Sheng M, Sabatini BL and Südhof TC. Synapses and Alzheimer's disease. *Cold Spring Harb Perspect Biol* 2012; 4: a005777.
66. Heneka MT, Carson MJ, Khoury JE, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015; 14: 388–405.
67. Nixon RA and Yang D-S. Autophagy failure in Alzheimer's disease—locating the primary defect. *Neurobiol Dis* 2011; 43: 38–45.
68. Oakley DH, Klickstein N, Commins C, et al. Continuous monitoring of tau-induced neurotoxicity in patient-derived iPSC-neurons. *J Neurosci* 2021; 41: 4335–4348.
69. Hardy J and Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002; 297: 353–356.
70. LaFerla FM, Green KN and Oddo S. Intracellular amyloid-β in Alzheimer's disease. *Nat Rev Neurosci* 2007; 8: 499–509.
71. Hung SY and Fu WM. Drug candidates in clinical trials for Alzheimer's disease. *J Biomed Sci* 2017; 24: 47. 20170719.
72. Manczak M, Anekonda TS, Henson E, et al. Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum Mol Genet* 2006; 15: 1437–1449.
73. Manczak M, Calkins MJ and Reddy PH. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. *Hum Mol Genet* 2011; 20: 2495–2509.
74. Calkins MJ, Manczak M, Mao P, et al. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Hum Mol Genet* 2011; 20: 4515–4529.
75. Reddy PH, Yin X, Manczak M, et al. Mutant APP and amyloid beta-induced defective autophagy, mitophagy, mitochondrial structural and functional changes and synaptic damage in hippocampal neurons from Alzheimer's disease. *Hum Mol Genet* 2018; 27: 2502–2516.
76. Kuruva CS, Manczak M, Yin X, et al. Aqua-soluble DDQ reduces the levels of Drp1 and Aβ and inhibits abnormal interactions between Aβ and Drp1 and protects Alzheimer's disease neurons from Aβ- and Drp1-induced mitochondrial and synaptic toxicities. *Hum Mol Genet* 2017; 26: 3375–3395.
77. Kumar S and Reddy PH. MicroRNA-455-3p as a potential biomarker for Alzheimer's disease: an update. *Front Aging Neurosci* 2018; 10: 41.
78. Islam MA, Sultana OF, Bandari M, et al. MicroRNA-455–3P as a peripheral biomarker and therapeutic target for mild cognitive impairment and Alzheimer's disease. *Ageing Res Rev* 2024; 100: 102459.