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Minimalistic *in vitro* systems for investigating tau pathology.

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Highlights

- Misfolded tau spreads from cell to cell, and propagates in a prion-like manner.
- *In vitro* and *in vivo* models exist that recapitulate aspects of tau pathology.
- Microfluidic devices can recreate minimalistic, manipulatable neuronal arrays *in vitro.*
- These devices have led to significant findings in the field of tauopathy research, and further use may lead to more advancements in our understanding of disease.

Abstract

Neurofibrillary tangles, formed of hyperphosphorylated, misfolded tau accumulations, are a pathological hallmark of neurodegenerative diseases such as Alzheimer's disease (AD) and frontotemporal dementia. The neuroanatomical localisation of tau pathology in AD brains of different disease stages suggests that tau tangle pathology is spreading throughout the brain along connected neuronal circuits. Pathogenic tau can act as a prion-like seed, inducing the misfolding of native tau and leading to disease propagation throughout the brain. However, it is not yet fully understood how tau spreads between individual neurons or brain regions.

Here, we review the models for investigating tau propagation *in vitro*, and summarise the findings from key studies into the mechanisms of tau pathology propagation in disease.

Keywords

Alzheimer's disease, tauopathy, tau, prion-like propagation, microfluidic devices

Introduction

Alzheimer's disease (AD) is the most common form a dementia, a progressive neurodegenerative disease characterised by extensive memory loss[1]. Its pathological hallmarks are the accumulation of extracellular amyloid beta plaques and intracellular tau neurofibrillary tangles (NFTs)[2]. Although NFTs were identified by Alois Alzheimer over a century ago[3], it was not until 1984 that it was discovered that their major component was tau[4].

Tau protein is best described as a stabiliser of axonal microtubules through binding tubulin dimers[5]. However, *Mapt*$^{-/-}$ mice develop normally[6], immunodepletion of tau does not affect microtubule growth and dynamics in cultured neurons[7], tau's residence time on microtubules is in the millisecond range[8], and a recent study demonstrated that siRNA against tau leads to an increase in stable but decrease in labile microtubules[9], altogether highlighting that the cell biology of tau is not yet fully understood. As well as binding to microtubules, tau has also been shown to anchor enzymes to microtubules, such as the phosphatases PP2A[5] and PP1[10]. In the developing neuron, tau becomes localised to axons as the neuron begins polarising[11]. Healthy neurons have a greater concentration of tau in their axons than in their somatodendritic compartment, and trafficking of tau between the somatodendritic region and the axon is controlled by diffusion barriers in the axon initial segment[12]. Although tau is primarily an axonal protein, it does exist at low levels in synaptic spines, where it recruits the Src kinase protein fyn into the spines[13]. As fyn is needed for synaptic plasticity[14] and tau is needed to recruit fyn to the dendritic spine[13], it is suggested that tau may also function in synaptic plasticity. Tau is also seen within the nucleus, where it plays a role in DNA and RNA protection[15], and in chromosomal stability[16].

Tau belongs to the microtubule-associated protein (MAP) family, and is coded for by the *Mapt* gene. There are 6 isoforms of tau within the central nervous system that arise from alternative splicing of its pre-mRNA, ranging from 352 to 441 amino acids and with a molecular weight of between 45 and 65 kDa. The isoforms differ regarding the presence of either 3 (3R) or 4 (4R) microtubule binding repeat regions in the C-terminus, resulting from

the splicing in or out of exon 10, and the presence or absence of 1 or 2 inserts in the N-terminus, resulting from the splicing in or out of exons 2 and/or 3 (0N, 1N, or 2N)[17].
The longest isoform, 2N4R tau, has 85 putative phosphorylation sites[18]. In a healthy cell, tau protein is dynamically phosphorylated and dephosphorylated, allowing it to both bind microtubules and facilitate dynamic microtubule network growth[19]. Tau is an intrinsically disordered protein that adopts different conformations depending on its binding status[20], and shifts between a microtubule bound conformational state, and an unbound, soluble state[21,22].
However in AD, post-translational modifications of tau, such as hyperphosphorylation, give tau a gain of secondary structure, in that it converts tau from an intrinsically disordered protein into a misfolded and insoluble conformation which leads to a transition from random coil to β-sheet conformation[23]. The appearance of β-sheet structures within NFTs was discovered before tau was identified as the main constituent of NFTs[24], and it has since been determined through circular dichroism[25] and X-ray diffraction[26] that misfolded tau protein adopts a β-sheet conformation upon aggregation.
Hyperphosphorylated or misfolded tau monomers dimerise together, and their assembly progresses into tau oligomers, paired helical filaments, and neurofibrillary tangles[27]. In the field of tau biology, the terminology used in different studies for the various tau species and stages of aggregation is often ambiguous, without confirmation/description of the specific species used. Recommendations for terminology to annotate different tau assemblies have been outlined in a recent review[28]. Upon misfolding, tau can act as a prion-like seed, triggering the misfolding and subsequent aggregation of native tau[29–31]. Indeed, post-mortem studies described prion-like propagation of tau pathology spreading throughout the brain of AD patients. In early AD, tau pathology begins in the entorhinal cortex, and with time progresses through the hippocampal formation, and finally throughout the cortex in later stages of the disease[2]. NFTs follow a pattern of spread so consistent that the appearance of NFTs in specific brain regions is incorporated into the criteria for Braak staging AD[2]; it progresses along anterogradely connected brain circuits, traversing synaptically connected cells[31–33]. Both the amount and distribution of NFTs correlate with the severity and the duration of AD[34–37]. However, not all tau tangles are alike: it has been recently shown that different structural folds of tau, leading to morphologically distinct filaments, are observed in brains of AD and Pick's disease patients[38,39], and these differences in tau conformation may be contributing to the differing pathophysiologies seen across the tauopathies.
In order to propagate misfolding in a prion-like manner along neuronal circuits, mechanisms must exist through which tau seeds are secreted or released by cells, and internalised by

connected cells. Here, we review current *in vitro* methods for investigating tau pathology and propagation at the cellular and subcellular level, and the main findings that have been elucidated through these methods.

Models to investigate tau pathology *in vitro*

Recombinant tau

In order to study the properties of tau aggregation, a frequently used tool is recombinant tau protein, purified from *Escherichia Coli* bacteria. As misfolding or aggregation of tau rarely occurs at physiological conditions[40–42], the rate of misfolding and subsequent tau fibrillisation is often chemically accelerated. Addition of arachidonic acid[43,44], free fatty acids[45,46], polyglutamate[47], and heparin[48–51] have been shown to effectively increase the rate of tau fibrillisation in cell-free assays. Another method of inducing recombinant tau aggregation is the use of truncated or mutated tau isoforms. The core sequence for tau aggregation lies within the microtubule binding repeat domain of tau[38,52]. A truncated tau isoform consisting of the microtubule binding repeat domain alone is referred to as RD-tau, K18 (the microtubule binding repeat domain of 4R tau) or K19 (the microtubule binding repeat domain of 3R tau)[23]. It has been shown using recombinant tau protein that this region alone efficiently causes tau to dimerise and aggregate[53], is endocytosed by cells *in vitro*[54], and can transfer from neuron to neuron[33]. In addition, the P301L mutation in tau that is found in patients with frontotemporal dementia[55] results in tau fibril formation in absence of polymerisation-inducing agents[56]. For the *in vitro* investigation of tau propagation, the addition of recombinant tau oligomers and fibrils to cultured cells is frequently used to recapitulate the prion-like seeding capabilities of tau[57–61].

Exogenous expression of tau

In early development, only 0N3R tau is present, and this isoform has been dubbed ‘foetal tau’[62]. In the mature adult human brain, all 6 isoforms are present, at a balanced ratio of 3R:4R isoforms[63]. However, in AD this balance is disturbed and there is an increase in 4R isoforms, altering the ratio of 4R:3R to approximately 2:1[64]. Aggregates contain a mix of all tau isoforms, with more 4R tau found within aggregates than 3R, presumably due to the increased ratio of 4R tau within the AD brain[65]. Interestingly, this differs between tauopathies, as tau filaments in Pick’s disease appear to be selectively containing 3R tau[39].

In order to investigate the physiological and pathological properties of tau, a commonly used method is the transfection of plasmids encoding for AD-related proteins into cellular systems. This method offers advantages of a controllable environment that can be investigated and readily manipulated in both live and fixed conditions. Cell lines have been generated that transiently express mutated tau[66–68], which have led to the characterisation of phosphorylated and aggregated tau *in vitro*. Many of these cell lines lack endogenous tau as well as synaptic specialisations. Neuronal cell lines containing endogenous tau, such as SH-SY5Y and PC12 cells, can be differentiated into neuron-like phenotypes bearing neurites. These cells have also been transfected to express tau, and have facilitated studies on tau interactions within neurites[69]. However, these neurites do not possess axonal/dendritic differentiations. As tau propagation is thought to be trans-synaptic along anterograde circuits[31,32], research into mechanisms of propagation benefit from the use of fully differentiated neurons, such as iPSCs and primary neurons.

An example of plasmids that have been used to transfect primary neurons were developed by Karen Ashe's lab[70]. Using 0N4R tau as a template and mutating disease-associated serine/threonine phosphorylation sites of tau to glutamate, they created a tau construct that is pseudo-hyperphosphorylated, i.e. mimicking constitutive phosphorylation. Each of the 14 sites that have been mutated are sites that have been shown to be phosphorylated *in vitro* and in tau filaments[18]. With these plasmids, it was discovered that both pseudo-hyperphosphorylated tau and tau^{P301L} are unable to bind to microtubules[70,71], mislocalise into dendritic spines, and cause a synaptic dysfunction as shown by a decrease in miniature excitatory postsynaptic current amplitude, thus causing a postsynaptic deficit[70]. Pseudohyperphosphorylation of tau also increases its propensity to fibrillise and form thioflavin S-positive aggregates, indicating increased β-sheet content[20,72,73]. Exogenous expression of disease-related tau is therefore a useful tool to examine the underlying mechanisms of cellular dysfunction.

Tau aggregation biosensors

Cell-based tau aggregation sensors provide a mechanism of investigating tau seeding with high sensitivity *in vitro.* An example of such a system is the fluorescence resonance energy transfer (FRET) tau sensor cells engineered by the Diamond lab[66,74]. It consists of HEK293 cells which express RD-tau with a P301S mutation tagged with either CFP or YFP. Minimal background FRET signal is reported with co-expression of these two tau constructs within the

cells, however upon addition of tau seeds, such as recombinant tau[66] or AD-brain homogenate[75], a FRET signal is generated, showing the prion-like templating of tau and demonstrating a method of investigating properties of tau seeding. This system has been used to demonstrate the existence of different strains of pathogenic tau, and demonstrated a link between tau strain, regional vulnerability and toxicity *in vitro* and *in vivo*[75]. Further, addition of brain extracts from AD patients to this sensor system revealed that tau seeds not only exist in the brain regions of the corresponding patients' Braak stage, but are also detectable in the subsequent region along the Braak pathway that has not yet developed overt pathology[76,77]. Another tau sensor system is that of the tau split-luciferase. In this system, tau is fused to either the N- or C-terminal part of Gaussia luciferase, such that tau oligomer formation reconstitutes the enzyme and results in a bioluminescent signal[78]. This is a sensitive assay through which tau aggregation can be monitored, and has been used to show that tau dimerisation does not necessarily lead to pathogenic tau aggregation[78]. These sensor systems are thus valuable tools to better understand the ability of different seeds or tau strains to induce aggregation, as well as assay the bioactivity of potential aggregation inhibitors.

Patient-derived iPSCs

One of the biggest disadvantages of using rodent models or cultures to mimic AD processes lies in the biological differences between humans and rodents, including the fact that AD and other related dementias are human specific diseases, rendering rodent models a second-class alternative to human brains. Mice differentially express 3R tau neonatally, and switch to exclusively 4R tau from young adulthood onwards[79], thus will never form the mixed isoform filaments described above. Further, rodent models do not spontaneously form tangle pathology. Therefore, in order to induce pathology, such models rely on the overexpression of target genes, unlike the normal gene expression levels in human patients. In order to better recapitulate human pathophysiology, inducible pluripotent stem cells (iPSCs) can be generated from human samples such as skin biopsies. iPSCs can be derived from healthy people or patients afflicted with *Mapt* mutations, such as the P301L/S mutation found in FTD that results in the canonical tau misfolding of tauopathies[80], and differentiated into neurons *in vitro*. This provides genetically human neurons that are self-programmed to develop the disease pathology being studied. An example of this are iPSCs derived from patients with tau^{P301L} mutation, which show increased tau phosphorylation, increased excitability and develop thicker processes and varicosity-like structures that are not present in the control

neurons[81]. Similarly, increased tau phosphorylation has been detected in iPSCs derived from individuals carrying the N279K or V337M mutations[82].

However, an obstacle in using iPSCs for examination of tau pathology lies in the developmentally regulated differential splicing of tau isoforms[83,84]. This is currently being investigated in detail by long term culturing of human FTD patient derived iPSCs, and through studies investigating the developmental splice regulatory differences between control and FTD patient-derived iPSCs[83,85]. 3D culture models of iPSCs can be used to bypass the problem of developmentally regulated splicing because of the increased level of 4R adult tau isoforms when compared to 2D culture[86], and thus form a good platform to study tau pathology. In the 3D culture of iPSCs expressing familial AD mutations, Gallyas-positive depositions and sarkosyl-resistant tau aggregates have been found, indicating the presence of neurofibrillary tangles[86]. Additionally, the ability to mutate *MAPT* in iPSCs via targeted genome editing facilitates the study of tau mutations in iPSCs[85]. This enables investigating the effect of a mutation in isogenic lines, excluding any contribution of other genetic variations. This technique has been used to study tauopathies related to the presence of 4R tau isoforms, as introducing the IVS10+16 mutation promotes the inclusion of exon 10[85]. When this mutation is introduced next to the P301S mutation, increased frequency of calcium bursts and increased tau oligomerisation upon seeding with pro-aggregant K18 fragments has been observed[85]. This may open up exciting new possibilities for a human platform of investigating tau propagation *in vitro.*

Investigating tau release *in vitro.*

The main discoveries in tau propagation *in vitro* are summarised below in Table 1.

In order for tau to spread from cell to cell and propagate misfolding of native tau, it must first be released from an initially affected cell. The transcellular tau spread in AD was initially thought to be due to the release of NFTs from dying cells into the extracellular space, from where they could have a toxic effect or spread to neighbouring cells[87]. However, as tau is found in human cerebrospinal fluid in both healthy and AD affected individuals[88], it is now thought that tau release is a physiological process. Indeed, it has been determined that native tau spreads from neuron to neuron *in vitro*[89], and that physiological tau is released by neurons in response to neuronal stimulation[90]. Therefore, there must exist other physiological mechanisms through which tau seeds are released from cells.

Two independent studies in 2012 by Chai et al. and Karch et al. both showed that 2N4R tau is secreted constitutively from HEK293 cells at low levels[91,92]. This tau was shown to be vesicle

independent and was suggested to be released as part of an unconventional secretion pathway. Other studies have examined the release of tau in an exosome-associated manner, a known mechanism of release of other aggregated proteins such as Aβ and α-synuclein[93–95], and have shown that tau protein is secreted in exosomes from M1C human neuroblastoma cells[96]. Tau can be both released freely and in microvesicles or exosomes by HEK293 cells and COS-7 cells[97,98]. The difference in release mechanism is suggested to be isoform specific – with 2N4R tau freely released, and 0N3R tau released in exosomes or microvesicles from both HEK and COS cell lines[91,98]. However, it has been suggested that tau is spread trans-synaptically[31–33], and HEK, COS and neuroblastoma cells do not possess the synaptic specialisations that could be responsible in part for the spread of pathogenic tau, meaning this evidence may not be directly translatable to neuronal cells. In addition to this, these cell lines employ constitutive exocytosis, whereas neuronal cells use both constitutive and regulated exocytosis, therefore the mechanisms of tau secretion could differ between cellular systems. Data from primary neurons and differentiated iPSCs has shown that c-terminally truncated tau is released from both cell types independent of cell death[99]. In primary neurons, tau release has been shown to be increased with activity[90,100] and it has recently been shown that Rab7a[101] and Rab1a[102] are involved in the modulation of tau release *in vitro*. In support of these observations suggesting synaptic release from intact neurons, a recent *in vivo* study showed that tau spread can occur before overt synaptic degeneration[103]. Additionally, it has been shown that tau associates with synaptic vesicles[104], further implicating a role of tau in the presynapse. However, it has not yet been explicitly confirmed if tau spread is synaptic, and to date the exact mechanism of release and interneuronal propagation of tau misfolding is not fully understood.

Investigating tau internalisation *in vitro*.

When tau is released by the cell into the extracellular space, there must be a mechanism in place by which neighbouring cells can internalise it. In order to act as a template to other tau proteins, thus propagating misfolding, newly internalised tau protein needs to access the cytosol. Indeed, misfolded tau from clarified brain lysates of mice transgenic for tau^{P301L} has been shown to be taken up by primary neurons *in vitro*, and to spread the conformational change from neuron to neuron in a minimalistic connected circuit[105,106]. Although many studies have been undertaken in an attempt to elucidate the underlying mechanism[57,60,107,108], the results have so far been inconclusive. A proposed mechanism of uptake is that of macropinocytosis, an endocytosis process involving ruffling of the cell surface[109]. In this

context, tau was shown to be taken up by C17 mouse neural progenitor cells by the formation of massive vesicles called macropinosomes[60]. This process was dependent on heparan sulfate proteoglycans (HSPGs). HSPGs have recently been shown again to play a role in tau internalisation, as the knockdown of *Hs6st1* (a gene involved in HSPG synthesis) by CRISPR in H4 human neuroglioma cells and iPSCs resulted in a decreased tau uptake *in vitro*[110]. This study also demonstrated a similar decrease in tau uptake upon knockdown of dynamin[110,111], a large GTPase involved in endocytosis[112]. Tau aggregates were further shown to colocalise with the fluid-phase uptake marker dextran in C17 cells, suggesting that aggregates also enter these cells through engulfment by the membrane in an endocytic pathway[57]. In agreement with this, misfolded tau was shown to be internalised by endocytosis by primary neurons, and to colocalise with dextran and Rab5[108], a small GTPase on the surface of early endosomes[113]. This suggests roles for endocytic mechanisms in both dividing and primary cells, following release of free tau into the extracellular space. An alternate method of tau spread from cell to cell without its entry into the extracellular space is through tunnelling nanotubes (TNTs). TNTs have been shown to transfer α-synuclein seeds between cells *in vitro*[114,115], and tau has been found to be present in TNTs connecting primary neurons[116]. Overall, the body of evidence shows a complex picture indicating multiple mechanisms that may be acting in concert. Additionally, varying mechanisms may be employed by different tau species and/or isoforms, so further investigation into these mechanisms may provide new insights to the pathway(s) of tau internalisation.

Table 1. Main findings from in vitro tau propagation studies.

Models of tauopathy	Advantages	Limitations	Main findings
Non-neuronal • CHO • HEK • C17 • COS	Dividing and immortal	Non-neuronal in origin No regulated exocytosis No neurites No synaptic specialisations	Tau secreted in exosomes[96] Tau secreted constitutively[91,92] Tau internalised through endocytosis[57] Tau internalised through macropinocytosis involving HSPGs[60,110]
Neuronal • SHSY-5Y • PC12	Dividing and immortal Can be differentiated to contain neurites Have regulated exocytosis	No axon/ dendrites specification No synaptic specialisations[33]	Monomers sufficient for propagation[54]
Stem cells • iPSCs • Mouse/human embryonic stem cells	Axon/dendrite specification Synaptic specialisations	Prolonged culture periods required for mature tau isoform expression	HSPGs involved in uptake[110]
Primary neurons	Axon/dendrite specification Synaptic specialisations	Difficult to assay directionality	Physiological tau is secreted in response to activity[90]
Minimalistic networks of primary neurons	Oriented and compartmentalised	Low throughput	Tau uptake through endocytosis[108] Tau uptake is size dependent[105,108] Tau propagation increased with synaptic connections[33] Pathogenic tau is secreted in response to activity[100] Physiological tau spreads from cell to cell[89]

Minimalistic *in vitro* compartments to study tau propagation.

Neuronal networks are highly complex, interconnected and compartmentalised circuits, which are difficult to recapitulate *in vitro*. Several of the aforementioned studies and their results were obtained through dissociated cell cultures, which lack the spatially organised connections seen *in vivo.* Microfluidic devices provide a method through which neuronal cultures can be arrayed and compartmentalised (Fig. 1) to recreate aspects of the ordered connectivity found *in vivo.* Such systems were first developed by Taylor et al., who designed a device to compartmentalise axonal outgrowths from the somatodendritic region of cultured neurons[117]. These devices consist of two cell culture channels, which are connected by a series of microchannels, through which neurites can extend. With sufficiently long outgrowth microchannels (>450μm), the growth of dendrites from one channel to the next can be prevented[117], rendering only axons capable of projecting from one chamber to the connecting chamber. With this, neurons can be cultured in one side of the device, the somatodendritic compartment, and these neurons project their axon and its presynaptic terminals into the other side of the device, the axonal compartment. The two compartments can be fluidically isolated by establishing a volume difference between them[117,118] – creating a flow that exceeds diffusion timescales to prevent the cross-talk by molecular diffusion between compartments. As the compartments can be fluidically isolated either side of the device can be treated and studied independently, thereby enabling the study of a variety of molecular processes, for example both retrograde and anterograde trafficking. In addition to this, microfluidic devices provide the user the ability to visualise neurons at single cell resolution, including detailed subcompartmental analysis. Using this concept and similar dimensions, further devices have been developed by labs investigating neuronal processes *in vitro*, and are summarised in Table 2.

Microfluidic devices isolate axonal processes from the somatodendritic region, and recreate a more ordered array of neurons *in vitro*, facilitating directionalised axonal transport studies. An early study using microfluidic devices for investigating the effects of tau expression on axonal transport found a differential effect of 3 and 4R tau isoforms on mitochondrial movement into axons of primary neurons overexpressing tau[119].

Using microfluidic devices to isolate the axon terminals from the somatodendritic region, it was shown that exogenously added tau is internalised both somatodendritically and axonally, and transported both anterogradely and retrogradely within the axon[108]. In this setting, only small tau species, and not larger fibrils, are internalised by neurons through endocytosis[108]. In

agreement with this, tau monomers and oligomers, but not tau fibrils, are endocytosed by iPSCs within microfluidic devices[120].

Microfluidic devices can also be used to study tau transfer between connected sets of cells, as well as the propagation of misfolding to the native tau. When two or more cell culture channels of a microfluidic device are loaded with cells the transfer and propagation of tau from cell to cell can be monitored. Co-culture neuronal setups have demonstrated that pathogenic tau seeded from exogenous seeds transfers from neuron to neuron[100]. Addition of tau fibrils isolated from mutant tau (K18-P301L) expressing HEK cells onto primary neurons revealed that transfer and propagation of labelled tau aggregates to neurons in the adjacent culture channel is enhanced when neurons are synaptically connected[33]. Consistent with this finding, exosomes derived from tau-expressing N2a mouse neuroblastoma cells are internalised and trafficked from cell to cell when added to primary neurons, but only when the neurons were synaptically connected within the devices[121]. This evidence suggests that tau transfer and propagation occur trans-synaptically.

High molecular weight tau (>670 kDa) is more readily taken up into primary neurons cultured in microfluidic devices and transferred through a connected cell circuit than low molecular weight tau[105]. In addition to this, adding a tau-specific antibody to the channel within which neurons are synaptically connected decreases the propagation of tau from cell to cell[67], highlighting free release of tau and a potential therapeutic avenue for tauopathy.

In the aforementioned studies of tau transfer and propagation, research groups expressed mutant tau within neurons, and additionally added exogenous tau seeds to trigger tau aggregation and propagation (Table 2). We recently showed that expression of mutant tau^{E14} or tau^{P301L} alone is sufficient to induce tau aggregation (our unpublished observation).

Table 2. Key findings from different tau seeding experiments in primary cultures.

Tau seed	Cell type	Main findings
Recombinant tau	Primary neurons	Internalised tau colocalises with dextran and Rab5[108]
		Internalised tau is transported bidirectionally[108]
		Uptake is specific to tau size[105,108]
	iPSCs	Monomer and oligomer, but not fibrils, internalised[120]
		Internalised tau causes neurodegeneration[120]
Cell-derived tau seeds	Primary neurons expressing tau^{P301L}	Synaptic contacts enhance tau propagation[33]
		BIN1 modulates tau propagation[122]
	Primary neurons expressing RD-tau	Tau propagation is enhanced with neuronal activity[100]
	Primary neurons	Tau propagation occurs in exosomes[121]
Transgenic brain extract	Primary neurons expressing $tau^{RD/P301L}$	Uptake is specific to tau size[105]
		Tau antibodies decrease uptake[106]
Lentiviral tau	Primary neurons	Wild type tau spreads from cell to cell[89]
Plasmid tau expression	Primary neurons	Misfolded tau propagates with high efficiency from neuron to neuron (unpublished observation)

Conclusions

In order to successfully develop disease modifying therapeutics for AD and other tauopathies, it is critical to understand the physiological role of tau, as well as the pathological mechanisms through which misfolded and/or hyperphosphorylated tau causes degeneration. If we understand the cellular mechanisms through which pathogenic tau is spreading and propagating, we have the potential to design therapeutics to delay, or prevent, the dissemination of tau pathology. In our quest to understand such mechanisms, *in vitro* platforms such as microfluidic devices can play a pivotal role in furthering our understanding of the cellular processes at work. This is because of the ability to follow entire individual neurons at single cell resolution over time *in vitro,* and also the ability to manipulate mechanisms at subcompartment specific regions. Current *in vitro* and microfluidic device

setups do not recapitulate the full brain environment, for example lacking a glial population or interplay with the immune system, and it has been shown that microglia can phagocytose tau aggregates and thus contribute to the dissemination of tau pathology. Depleting microglia from two different transgenic mice models, one expressing tau^{P301L} and the second expressing tau^{P301S}, suppressed tau pathology propagation[123]. Astrocytes have also been shown to be able to release tau and phosphorylated tau in exosomes[124]. These examples suggest that generating the system mirroring the brain environment with all its cell types is indispensable to fully understand tau spread. Further development of co-cultures may therefore enhance our understanding of the synergy between these systems. Additionally, technical developments of microfluidic device designs are increasing our capabilities to orientate the synaptic junction and therefore recreate a unidirectional neuronal circuit[125,126], which may further our understanding into the directionalised propagation of tau and other prion-like proteins. With these and other *in vitro* models, further investigations into differing tau species, mechanisms of propagation and inhibitors of these mechanisms, could potentially facilitate the development of new therapeutics for AD.

Competing interests

The authors have no competing interests to declare.

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References

1. National Institute on Aging. Alzheimer's Disease Fact Sheet. https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet. Published 2018. Accessed July 22, 2018.
2. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82(4):239-259. http://www.ncbi.nlm.nih.gov/pubmed/1759558. Accessed March 1, 2015.
3. Alzheimer A. Über eine eigenartige Erkrankung der Hirnrinde. *Allg Z Psychiatr Psych Med*. 1907;64:146-148.
4. Kosik KS, Duffy LK, Dowling MM, Abraham C, McCluskey A, Selkoe DJ. Microtubule-associated protein 2: monoclonal antibodies demonstrate the selective incorporation of certain epitopes into Alzheimer neurofibrillary tangles. *Proc Natl Acad Sci U S A*. 1984;81(24):7941-7945. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=392269&tool=pmcentrez&rendertype=abstract. Accessed March 24, 2015.
5. Sontag E, Nunbhakdi-Craig V, Lee G, et al. Molecular interactions among protein phosphatase 2A, tau, and microtubules. Implications for the regulation of tau phosphorylation and the development of tauopathies. *J Biol Chem*. 1999;274(36):25490-25498. http://www.ncbi.nlm.nih.gov/pubmed/10464280. Accessed March 24, 2015.
6. Harada A, Oguchi K, Okabe S, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature*. 1994;369(6480):488-491. doi:10.1038/369488a0
7. Tint I, Slaughter T, Fischer I, Black MM. Acute inactivation of tau has no effect on dynamics of microtubules in growing axons of cultured sympathetic neurons. *J Neurosci*. 1998;18(21):8660-8673. doi:10.1523/JNEUROSCI.18-21-08660.1998
8. Janning D, Igaev M, Sündermann F, et al. Single-molecule tracking of tau reveals fast kiss-and-hop interaction with microtubules in living neurons. Lippincott-Schwartz J, Lippincott-Schwartz J, eds. *Mol Biol Cell*. 2014;25(22):3541-3551. doi:10.1091/mbc.e14-06-1099
9. Qiang L, Sun X, Austin TO, et al. Tau Does Not Stabilize Axonal Microtubules but Rather Enables Them to Have Long Labile Domains. *Curr Biol*. 2018;28(13):2181-2189.e4. doi:10.1016/J.CUB.2018.05.045
10. Liao H, Li Y, Brautigan DL, Gundersen GG. Protein Phosphatase 1 Is Targeted to

Microtubules by the Microtubule-associated Protein Tau. *J Biol Chem*. 1998;273(34):21901-21908. doi:10.1074/jbc.273.34.21901

11. Kempf M, Clement A, Faissner A, Lee G, Brandt R. Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner. *J Neurosci*. 1996;16(18):5583-5592. http://www.ncbi.nlm.nih.gov/pubmed/8795614. Accessed March 24, 2015.
12. Zempel H, Dennissen FJA, Kumar Y, et al. Axodendritic sorting and pathological missorting of Tau are isoform-specific and determined by axon initial segment architecture. *J Biol Chem*. 2017;292(29):12192-12207. doi:10.1074/jbc.M117.784702
13. Ittner LM, Ke YD, Delerue F, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell*. 2010;142(3):387-397. doi:10.1016/j.cell.2010.06.036
14. Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science*. 1992;258(5090):1903-1910. http://www.ncbi.nlm.nih.gov/pubmed/1361685. Accessed August 31, 2017.
15. Violet M, Delattre L, Tardivel M, et al. A major role for Tau in neuronal DNA and RNA protection in vivo under physiological and hyperthermic conditions. *Front Cell Neurosci*. 2014;8:84. doi:10.3389/fncel.2014.00084
16. Rossi G, Dalprà L, Crosti F, et al. A new function of microtubule-associated protein tau: Involvement in chromosome stability. *Cell Cycle*. 2008;7(12):1788-1794. doi:10.4161/cc.7.12.6012
17. Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev*. 2000;33(1):95-130. http://www.ncbi.nlm.nih.gov/pubmed/10967355. Accessed January 12, 2015.
18. Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med*. 2009;15(3):112-119. doi:10.1016/j.molmed.2009.01.003
19. Rodríguez-Martín T, Cuchillo-Ibáñez I, Noble W, Nyenya F, Anderton BH, Hanger DP. Tau phosphorylation affects its axonal transport and degradation. *Neurobiol Aging*. 2013;34(9):2146-2157. doi:10.1016/j.neurobiolaging.2013.03.015
20. Jeganathan S, Hascher A, Chinnathambi S, Biernat J, Mandelkow E-M, Mandelkow E. Proline-directed pseudo-phosphorylation at AT8 and PHF1 epitopes induces a

compaction of the paperclip folding of Tau and generates a pathological (MC-1) conformation. *J Biol Chem*. 2008;283(46):32066-32076. doi:10.1074/jbc.M805300200

21. Cleveland DW, Hwo SY, Kirschner MW. Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin. *J Mol Biol*. 1977;116(2):207-225. http://www.ncbi.nlm.nih.gov/pubmed/599557. Accessed March 24, 2015.
22. Meraz-Ríos MA, Lira-De León KI, Campos-Peña V, De Anda-Hernández MA, Mena-López R. Tau oligomers and aggregation in Alzheimer's disease. *J Neurochem*. 2010;112(6):1353-1367. doi:10.1111/j.1471-4159.2009.06511.x
23. von Bergen M, Barghorn S, Biernat J, Mandelkow E-M, Mandelkow E. Tau aggregation is driven by a transition from random coil to beta sheet structure. *Biochim Biophys Acta - Mol Basis Dis*. 2005;1739(2-3):158-166. doi:10.1016/j.bbadis.2004.09.010
24. Kirschner DA, Abraham C, Selkoe DJ. X-ray diffraction from intraneuronal paired helical filaments and extraneuronal amyloid fibers in Alzheimer disease indicates cross-beta conformation. *Proc Natl Acad Sci U S A*. 1986;83(2):503-507. http://www.ncbi.nlm.nih.gov/pubmed/3455785. Accessed September 1, 2017.
25. Barghorn S, Zheng-Fischhöfer Q, Ackmann M, et al. Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias. *Biochemistry*. 2000;39(38):11714-11721. http://www.ncbi.nlm.nih.gov/pubmed/10995239. Accessed February 25, 2015.
26. Giannetti AM, Lindwall G, Kohlstaedt LA, Chau M-F, Radeke MJ, Feinstein SC. Fibers of tau fragments, but not full length tau, exhibit a cross β-structure: Implications for the formation of paired helical filaments. *Protein Sci*. 2000;9(12):2427-2435. doi:10.1110/ps.9.12.2427
27. Cowan CM, Mudher A. Are tau aggregates toxic or protective in tauopathies? *Front Neurol*. 2013;4:114. doi:10.3389/fneur.2013.00114
28. Mudher A, Colin M, Dujardin S, et al. What is the evidence that tau pathology spreads through prion-like propagation? *Acta Neuropathol Commun*. 2017;5(1):99. doi:10.1186/s40478-017-0488-7
29. Sanders DW, Kaufman SK, DeVos SL, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron*. 2014;82(6):1271-1288. doi:10.1016/j.neuron.2014.04.047
30. Clavaguera F, Akatsu H, Fraser G, et al. Brain homogenates from human tauopathies

induce tau inclusions in mouse brain. *Proc Natl Acad Sci U S A*. 2013;110(23):9535-9540. doi:10.1073/pnas.1301175110

31. de Calignon A, Polydoro M, Suárez-Calvet M, et al. Propagation of Tau Pathology in a Model of Early Alzheimer's Disease. *Neuron*. 2012;73(4):685-697. doi:10.1016/j.neuron.2011.11.033
32. Liu L, Drouet V, Wu JW, et al. Trans-Synaptic Spread of Tau Pathology In Vivo. Ikezu T, ed. *PLoS One*. 2012;7(2):e31302. doi:10.1371/journal.pone.0031302
33. Calafate S, Buist A, Miskiewicz K, et al. Synaptic Contacts Enhance Cell-to-Cell Tau Pathology Propagation. *Cell Rep*. 2015;11(8):1176-1183. doi:10.1016/j.celrep.2015.04.043
34. Arriagada P V, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology*. 1992;42(3 Pt 1):631-639. http://www.ncbi.nlm.nih.gov/pubmed/1549228. Accessed February 13, 2015.
35. Gómez-Isla T, Hollister R, West H, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. 1997;41(1):17-24. doi:10.1002/ana.410410106
36. Giannakopoulos P, Herrmann FR, Bussière T, et al. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology*. 2003;60(9):1495-1500. http://www.ncbi.nlm.nih.gov/pubmed/12743238. Accessed March 24, 2015.
37. Ingelsson M, Fukumoto H, Newell KL, et al. Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. *Neurology*. 2004;62(6):925-931. http://www.ncbi.nlm.nih.gov/pubmed/15037694. Accessed March 24, 2015.
38. Fitzpatrick AWP, Falcon B, He S, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature*. 2017;547(7662):185-190. doi:10.1038/nature23002
39. Falcon B, Zhang W, Murzin AG, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature*. 2018;561(7721):137-140. doi:10.1038/s41586-018-0454-y
40. Wilson DM, Binder LI. Polymerization of microtubule-associated protein tau under near-physiological conditions. *J Biol Chem*. 1995;270(41):24306-24314. http://www.ncbi.nlm.nih.gov/pubmed/7592641. Accessed July 24, 2018.
41. Barghorn S, Biernat J, Mandelkow E. Purification of recombinant tau protein and

preparation of Alzheimer-paired helical filaments in vitro. *Methods Mol Biol*. 2005;299:35-51. http://www.ncbi.nlm.nih.gov/pubmed/15980594. Accessed July 24, 2018.

42. Wille H, Drewes G, Biernat J, Mandelkow EM, Mandelkow E. Alzheimer-like paired helical filaments and antiparallel dimers formed from microtubule-associated protein tau in vitro. *J Cell Biol*. 1992;118(3):573-584. http://www.ncbi.nlm.nih.gov/pubmed/1639844. Accessed July 24, 2018.
43. King ME, Gamblin TC, Kuret J, Binder LI. Differential assembly of human tau isoforms in the presence of arachidonic acid. *J Neurochem*. 2000;74(4):1749-1757. http://www.ncbi.nlm.nih.gov/pubmed/10737634. Accessed July 24, 2018.
44. T. Chris Gamblin *,‡, Michelle E. King ‡, Hana Dawson §, et al. In Vitro Polymerization of Tau Protein Monitored by Laser Light Scattering: Method and Application to the Study of FTDP-17 Mutants†. 2000. doi:10.1021/BI000201F
45. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol*. 1997;150(6):2181-2195. http://www.ncbi.nlm.nih.gov/pubmed/9176408. Accessed July 24, 2018.
46. Chirita CN, Necula M, Kuret J. Anionic micelles and vesicles induce tau fibrillization in vitro. *J Biol Chem*. 2003;278(28):25644-25650. doi:10.1074/jbc.M301663200
47. Friedhoff P, Schneider A, Mandelkow E-M, Mandelkow E. Rapid Assembly of Alzheimer-like Paired Helical Filaments from Microtubule-Associated Protein Tau Monitored by Fluorescence in Solution †. *Biochemistry*. 1998;37(28):10223-10230. doi:10.1021/bi980537d
48. Xu S, Brunden KR, Trojanowski JQ, Lee VM-Y. Characterization of tau fibrillization in vitro. *Alzheimers Dement*. 2010;6(2):110-117. doi:10.1016/j.jalz.2009.06.002
49. Pérez M, Valpuesta JM, Medina M, Montejo de Garcini E, Avila J. Polymerization of tau into filaments in the presence of heparin: the minimal sequence required for tau-tau interaction. *J Neurochem*. 1996;67(3):1183-1190. http://www.ncbi.nlm.nih.gov/pubmed/8752125. Accessed July 24, 2018.
50. Sahara N, Maeda S, Murayama M, et al. Assembly of two distinct dimers and higher-order oligomers from full-length tau. *Eur J Neurosci*. 2007;25(10):3020-3029. doi:10.1111/j.1460-9568.2007.05555.x
51. Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ, Crowther RA. Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced

by sulphated glycosaminoglycans. *Nature*. 1996;383(6600):550-553. doi:10.1038/383550a0

52. von Bergen M, Barghorn S, Li L, et al. Mutations of tau protein in frontotemporal dementia promote aggregation of paired helical filaments by enhancing local beta-structure. *J Biol Chem*. 2001;276(51):48165-48174. doi:10.1074/jbc.M105196200
53. Fischer D, Mukrasch MD, von Bergen M, et al. Structural and microtubule binding properties of tau mutants of frontotemporal dementias. *Biochemistry*. 2007;46(10):2574-2582. doi:10.1021/bi061318s
54. Michel CH, Kumar S, Pinotsi D, et al. Extracellular monomeric tau protein is sufficient to initiate the spread of tau protein pathology. *J Biol Chem*. 2014;289(2):956-967. doi:10.1074/jbc.M113.515445
55. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998;393(6686):702-705. doi:10.1038/31508
56. Nacharaju P, Lewis J, Easson C, et al. Accelerated filament formation from tau protein with specific FTDP-17 missense mutations. *FEBS Lett*. 1999;447(2-3):195-199. doi:10.1016/S0014-5793(99)00294-X
57. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem*. 2009;284(19):12845-12852. doi:10.1074/jbc.M808759200
58. Mirbaha H, Holmes BB, Sanders DW, Bieschke J, Diamond MI. Tau Trimers Are the Minimal Propagation Unit Spontaneously Internalized to Seed Intracellular Aggregation. *J Biol Chem*. 2015;290(24):14893-14903. doi:10.1074/jbc.M115.652693
59. Falcon B, Cavallini A, Angers R, et al. Conformation determines the seeding potencies of native and recombinant Tau aggregates. *J Biol Chem*. 2015;290(2):1049-1065. doi:10.1074/jbc.M114.589309
60. Holmes BB, DeVos SL, Kfoury N, et al. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci U S A*. 2013;110(33):E3138-47. doi:10.1073/pnas.1301440110
61. Guo JL, Lee VM-Y. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem*. 2011;286(17):15317-15331. doi:10.1074/jbc.M110.209296
62. Kosik KS, Orecchio LD, Bakalis S, Neve RL. Developmentally regulated expression of specific tau sequences. *Neuron*. 1989;2(4):1389-1397. doi:10.1016/0896-

6273(89)90077-9

63. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*. 1989;3(4):519-526. http://www.ncbi.nlm.nih.gov/pubmed/2484340. Accessed April 8, 2015.
64. Ginsberg SD, Che S, Counts SE, Mufson EJ. Shift in the ratio of three-repeat tau and four-repeat tau mRNAs in individual cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease. *J Neurochem*. 2006;96(5):1401-1408. doi:10.1111/j.1471-4159.2005.03641.x
65. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998;393(6686):702-705. doi:10.1038/31508
66. Holmes BB, Furman JL, Mahan TE, et al. Proteopathic tau seeding predicts tauopathy in vivo. *Proc Natl Acad Sci U S A*. 2014;111(41):E4376-85. doi:10.1073/pnas.1411649111
67. Knops J, Kosik KS, Lee G, Pardee JD, Cohen-Gould L, McConlogue L. Overexpression of tau in a nonneuronal cell induces long cellular processes. *J Cell Biol*. 1991;114(4):725-733. http://www.ncbi.nlm.nih.gov/pubmed/1678391. Accessed September 3, 2017.
68. Vogelsberg-Ragaglia V, Bruce J, Richter-Landsberg C, et al. Distinct FTDP-17 missense mutations in tau produce tau aggregates and other pathological phenotypes in transfected CHO cells. *Mol Biol Cell*. 2000;11(12):4093-4104. http://www.ncbi.nlm.nih.gov/pubmed/11102510. Accessed September 3, 2017.
69. Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol*. 1998;143(3):777-794. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2148132&tool=pmcentrez&rendertype=abstract. Accessed June 29, 2015.
70. Hoover BR, Reed MN, Su J, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron*. 2010;68(6):1067-1081. doi:10.1016/j.neuron.2010.11.030
71. Sun Q, Gamblin TC. Pseudohyperphosphorylation causing AD-like changes in tau has significant effects on its polymerization. *Biochemistry*. 2009;48(25):6002-6011.

doi:10.1021/bi900602h

72. Fath T, Eidenmüller J, Brandt R. Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. *J Neurosci*. 2002;22(22):9733-9741. doi:10.1523/JNEUROSCI.22-22-09733.2002
73. Haase C, Stieler JT, Arendt T, Holzer M. Pseudophosphorylation of tau protein alters its ability for self-aggregation. *J Neurochem*. 2004;88(6):1509-1520. doi:10.1046/j.1471-4159.2003.02287.x
74. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of Tau aggregation by fibrillar species. *J Biol Chem*. 2012;287(23):19440-19451. doi:10.1074/jbc.M112.346072
75. Kaufman SK, Sanders DW, Thomas TL, et al. Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo. *Neuron*. 2016;92(4):796-812. doi:10.1016/j.neuron.2016.09.055
76. Kaufman SK, Del Tredici K, Thomas TL, Braak H, Diamond MI. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol*. 2018;136(1):57-67. doi:10.1007/s00401-018-1855-6
77. DeVos SL, Corjuc BT, Oakley DH, et al. Synaptic Tau Seeding Precedes Tau Pathology in Human Alzheimer's Disease Brain. *Front Neurosci*. 2018;12:267. doi:10.3389/fnins.2018.00267
78. Wegmann S, Nicholls S, Takeda S, Fan Z, Hyman BT. Formation, release, and internalization of stable tau oligomers in cells. *J Neurochem*. 2016;139(6):1163-1174. doi:10.1111/jnc.13866
79. McMillan P, Korvatska E, Poorkaj P, et al. Tau isoform regulation is region- and cell-specific in mouse brain. *J Comp Neurol*. 2008;511(6):788-803. doi:10.1002/cne.21867
80. Yen S, Easson C, Nacharaju P, Hutton M, Yen S-H. FTDP-17 tau mutations decrease the susceptibility of tau to calpain I digestion. *FEBS Lett*. 1999;461(1-2):91-95. doi:10.1016/S0014-5793(99)01427-1
81. Iovino M, Agathou S, González-Rueda A, et al. Early maturation and distinct tau pathology in induced pluripotent stem cell-derived neurons from patients with *MAPT* mutations. *Brain*. 2015;138(11):3345-3359. doi:10.1093/brain/awv222
82. Ehrlich M, Hallmann A-L, Reinhardt P, et al. Distinct Neurodegenerative Changes in an Induced Pluripotent Stem Cell Model of Frontotemporal Dementia Linked to Mutant TAU Protein. *Stem cell reports*. 2015;5(1):83-96.

doi:10.1016/j.stemcr.2015.06.001

83. Sposito T, Preza E, Mahoney CJ, et al. Developmental regulation of tau splicing is disrupted in stem cell-derived neurons from frontotemporal dementia patients with the 10 + 16 splice-site mutation in MAPT. *Hum Mol Genet*. 2015;24(18):5260-5269. doi:10.1093/hmg/ddv246
84. Verheyen A, Diels A, Dijkmans J, et al. Using Human iPSC-Derived Neurons to Model TAU Aggregation. Avila J, ed. *PLoS One*. 2015;10(12):e0146127. doi:10.1371/journal.pone.0146127
85. Verheyen A, Diels A, Reumers J, et al. Genetically Engineered iPSC-Derived FTDP-17 MAPT Neurons Display Mutation-Specific Neurodegenerative and Neurodevelopmental Phenotypes. *Stem Cell Reports*. 2018;11(2):363-379. doi:10.1016/J.STEMCR.2018.06.022
86. Choi SH, Kim YH, Hebisch M, et al. A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature*. 2014;515(7526):274-278. doi:10.1038/nature13800
87. Gómez-Ramos A, Díaz-Hernández M, Cuadros R, Hernández F, Avila J. Extracellular tau is toxic to neuronal cells. *FEBS Lett*. 2006;580(20):4842-4850. doi:10.1016/j.febslet.2006.07.078
88. Arai H, Terajima M, Miura M, et al. Tau in cerebrospinal fluid: A potential diagnostic marker in Alzheimer's disease. *Ann Neurol*. 1995;38(4):649-652. doi:10.1002/ana.410380414
89. Dujardin S, Lécolle K, Caillierez R, et al. Neuron-to-neuron wild-type Tau protein transfer through a trans-synaptic mechanism: relevance to sporadic tauopathies. *Acta Neuropathol Commun*. 2014;2:14. doi:10.1186/2051-5960-2-14
90. Pooler AM, Phillips EC, Lau DHW, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep*. 2013;14(4):389-394. doi:10.1038/embor.2013.15
91. Chai X, Dage JL, Citron M. Constitutive secretion of tau protein by an unconventional mechanism. *Neurobiol Dis*. 2012;48(3):356-366. doi:10.1016/j.nbd.2012.05.021
92. Karch CM, Jeng AT, Goate AM. Extracellular Tau levels are influenced by variability in Tau that is associated with tauopathies. *J Biol Chem*. 2012;287(51):42751-42762. doi:10.1074/jbc.M112.380642
93. Emmanouilidou E, Melachroinou K, Roumeliotis T, et al. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts

neuronal survival. *J Neurosci*. 2010;30(20):6838-6851. doi:10.1523/JNEUROSCI.5699-09.2010

94. Rajendran L, Honsho M, Zahn TR, et al. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc Natl Acad Sci U S A*. 2006;103(30):11172-11177. doi:10.1073/pnas.0603838103
95. Fevrier B, Vilette D, Archer F, et al. Cells release prions in association with exosomes. *Proc Natl Acad Sci U S A*. 2004;101(26):9683-9688. doi:10.1073/pnas.0308413101
96. Saman S, Kim W, Raya M, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem*. 2012;287(6):3842-3849. doi:10.1074/jbc.M111.277061
97. Simón D, García-García E, Gómez-Ramos A, et al. Tau overexpression results in its secretion via membrane vesicles. *Neurodegener Dis*. 2012;10(1-4):73-75. doi:10.1159/000334915
98. Simón D, García-García E, Royo F, Falcón-Pérez JM, Avila J. Proteostasis of tau. Tau overexpression results in its secretion via membrane vesicles. *FEBS Lett*. 2012;586(1):47-54. doi:10.1016/j.febslet.2011.11.022
99. Kanmert D, Cantlon A, Muratore CR, et al. C-Terminally Truncated Forms of Tau, But Not Full-Length Tau or Its C-Terminal Fragments, Are Released from Neurons Independently of Cell Death. *J Neurosci*. 2015;35(30):10851-10865. doi:10.1523/JNEUROSCI.0387-15.2015
100. Wu JW, Hussaini SA, Bastille IM, et al. Neuronal activity enhances tau propagation and tau pathology in vivo. *Nat Neurosci*. 2016;19(8):1085-1092. doi:10.1038/nn.4328
101. Rodriguez L, Mohamed N-V, Desjardins A, Lippé R, Fon EA, Leclerc N. Rab7A regulates tau secretion. *J Neurochem*. 2017;141(4):592-605. doi:10.1111/jnc.13994
102. Mohamed N-V, Desjardins A, Leclerc N. Tau secretion is correlated to an increase of Golgi dynamics. Iijima KM, ed. *PLoS One*. 2017;12(5):e0178288. doi:10.1371/journal.pone.0178288
103. Pickett EK, Henstridge CM, Allison E, et al. Spread of tau down neural circuits precedes synapse and neuronal loss in the rTgTauEC mouse model of early Alzheimer's disease. *Synapse*. 2017;71(6):e21965. doi:10.1002/syn.21965
104. Zhou L, McInnes J, Wierda K, et al. Tau association with synaptic vesicles causes presynaptic dysfunction. *Nat Commun*. 2017;8:15295. doi:10.1038/ncomms15295
105. Takeda S, Wegmann S, Cho H, et al. Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain.

Nat Commun. 2015;6:8490. doi:10.1038/ncomms9490

106. Nobuhara CK, DeVos SL, Commins C, et al. Tau Antibody Targeting Pathological Species Blocks Neuronal Uptake and Interneuron Propagation of Tau in Vitro. *Am J Pathol*. 2017;187(6):1399-1412. doi:10.1016/j.ajpath.2017.01.022
107. Rauch JN, Chen JJ, Sorum AW, et al. Tau Internalization is Regulated by 6-O Sulfation on Heparan Sulfate Proteoglycans (HSPGs). *Sci Rep*. 2018;8(1):6382. doi:10.1038/s41598-018-24904-z
108. Wu JW, Herman M, Liu L, et al. Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem*. 2013;288(3):1856-1870. doi:10.1074/jbc.M112.394528
109. Swanson JA, Watts C. Macropinocytosis. *Trends Cell Biol*. 1995;5(11):424-428. http://www.ncbi.nlm.nih.gov/pubmed/14732047. Accessed March 24, 2015.
110. Rauch JN, Chen JJ, Sorum AW, et al. Tau Internalization is Regulated by 6-O Sulfation on Heparan Sulfate Proteoglycans (HSPGs). *Sci Rep*. 2018;8(1):6382. doi:10.1038/s41598-018-24904-z
111. Evans LD, Wassmer T, Fraser G, et al. Extracellular Monomeric and Aggregated Tau Efficiently Enter Human Neurons through Overlapping but Distinct Pathways. *Cell Rep*. 2018;22(13):3612-3624. doi:10.1016/j.celrep.2018.03.021
112. Muhlberg AB, Warnock DE, Schmid SL. Domain structure and intramolecular regulation of dynamin GTPase. *EMBO J*. 1997;16(22):6676-6683. doi:10.1093/emboj/16.22.6676
113. Gorvel JP, Chavrier P, Zerial M, Gruenberg J. Rab5 controls early endosome fusion in vitro. *Cell*. 1991;64(5):915-925. http://www.ncbi.nlm.nih.gov/pubmed/1900457. Accessed August 7, 2018.
114. Abounit S, Bousset L, Loria F, et al. Tunneling nanotubes spread fibrillar α-synuclein by intercellular trafficking of lysosomes. *EMBO J*. 2016;35(19):2120-2138. doi:10.15252/embj.201593411
115. Costanzo M, Abounit S, Marzo L, et al. Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes. *J Cell Sci*. 2013;126(16):3678-3685. doi:10.1242/jcs.126086
116. Tardivel M, Bégard S, Bousset L, et al. Tunneling nanotube (TNT)-mediated neuron-to neuron transfer of pathological Tau protein assemblies. *Acta Neuropathol Commun*. 2016;4(1):117. doi:10.1186/s40478-016-0386-4
117. Taylor AM, Rhee SW, Tu CH, Cribbs DH, Cotman CW, Jeon NL. Microfluidic

Multicompartment Device for Neuroscience Research. *Langmuir*. 2003;19(5):1551-1556. doi:10.1021/la026417v

118. Dinh N-D, Chiang Y-Y, Hardelauf H, et al. Microfluidic construction of minimalistic neuronal co-cultures. *Lab Chip*. 2013;13(7):1402-1412. doi:10.1039/c3lc41224e
119. Stoothoff W, Jones PB, Spires-Jones TL, et al. Differential effect of three-repeat and four-repeat tau on mitochondrial axonal transport. *J Neurochem*. 2009;111(2):417-427. doi:10.1111/j.1471-4159.2009.06316.x
120. Usenovic M, Niroomand S, Drolet RE, et al. Internalized Tau Oligomers Cause Neurodegeneration by Inducing Accumulation of Pathogenic Tau in Human Neurons Derived from Induced Pluripotent Stem Cells. *J Neurosci*. 2015;35(42):14234-14250. doi:10.1523/JNEUROSCI.1523-15.2015
121. Wang Y, Balaji V, Kaniyappan S, et al. The release and trans-synaptic transmission of Tau via exosomes. *Mol Neurodegener*. 2017;12(1):5. doi:10.1186/s13024-016-0143-y
122. Calafate S, Flavin W, Verstreken P, Moechars D. Loss of Bin1 Promotes the Propagation of Tau Pathology. *Cell Rep*. 2016;17(4):931-940. doi:10.1016/j.celrep.2016.09.063
123. Asai H, Ikezu S, Tsunoda S, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat Neurosci*. 2015;18(11):1584-1593. doi:10.1038/nn.4132
124. Chiarini A, Armato U, Gardenal E, Gui L, Dal Prà I. Amyloid β-Exposed Human Astrocytes Overproduce Phospho-Tau and Overrelease It within Exosomes, Effects Suppressed by Calcilytic NPS 2143-Further Implications for Alzheimer's Therapy. *Front Neurosci*. 2017;11:217. doi:10.3389/fnins.2017.00217
125. Renault R, Durand J-B, Viovy J-L, Villard C. Asymmetric axonal edge guidance: a new paradigm for building oriented neuronal networks. *Lab Chip*. 2016;16(12):2188-2191. doi:10.1039/c6lc00479b
126. Malishev E, Pimashkin A, Gladkov A, et al. Microfluidic device for unidirectional axon growth. *J Phys Conf Ser*. 2015;643(1):012025. doi:10.1088/1742-6596/643/1/012025

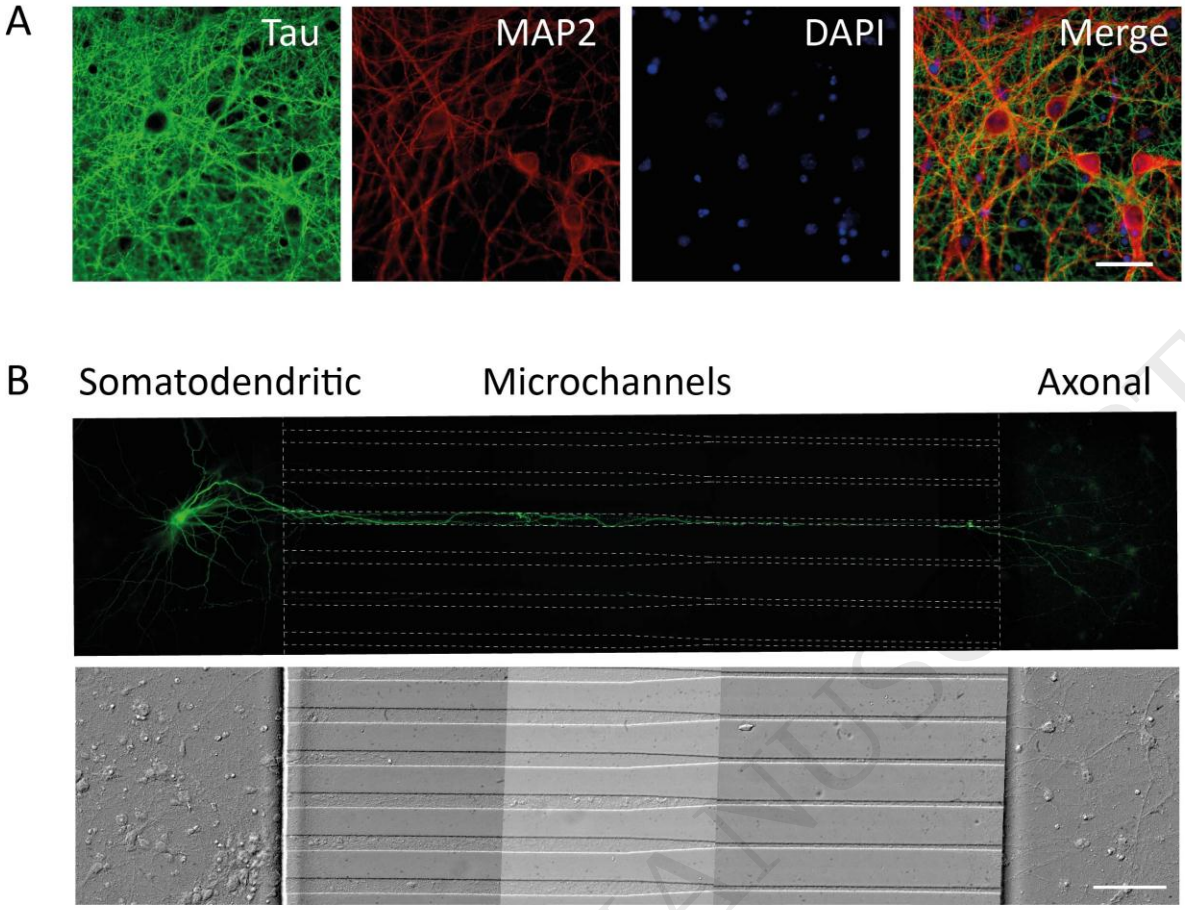

Figure 1. Mass culture and microfluidic device culture comparison. (a) Mass culture of primary hippocampal neurons form a highly interconnected network *in vitro*. Axonal (tau), dendritic (MAP2) and nuclear (DAPI) stain. Scale bar, 30 µm. (b) Microfluidic devices compartmentalise and array primary neurons. Image shows a neuron transfected with GFP-Tau (above), and the corresponding DIC image of the culture (below). Dashed lines indicate channel boundaries. Scale bar, 30 µm.