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■ REVIEW ARTICLE

In Situ Cellular Reprogramming: A Novel Targeting of Parkinson's Disease

Katherine Lau

Abbotsleigh, 1666 Pacific Hwy, Wahroongah, Sydney, NSW, 2076, Australia; kathykl1011@gmail.com

ABSTRACT: Parkinson's disease (PD) is estimated to affect around 8.5 million globally in 2019, according to the World Health Organization. Yet still, a cure for these people remains an unforeseeable dream. PD pathological hallmarks include the degeneration of dopaminergic neurons in the substantia nigra; however, the specific cause of this degeneration is unknown. It is believed to be caused by both genetic and environmental influences. Current therapies only offer symptomatic relief which inevitably worsens with age in tandem with PD degeneration. *In situ* cellular reprogramming (or the cell fate switch) differentiates itself from current treatments by targeting the root of PD symptoms: It replaces lost dopamine neurons in the substantia nigra and can reconstruct lost neural circuitry required for neuron function. The purpose of this review is to assess the translatability of in situ cellular reprogramming as a treatment for Parkinson's disease, as well as the benefits it provides in comparison to current therapies. This review also discusses future directions for research, guided by gaps in current knowledge, to shed light on possible approaches to curing PD.

KEYWORDS: Translational Medical Sciences, Disease Treatment and Therapies, *In Situ* Cellular Reprogramming, Parkinson's Disease, Dopaminergic Neurons.

■ Introduction

PD is identifiable by its pathological hallmarks: the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and the presence of Lewy bodies in neurons caused by α-synuclein aggregation (Figure 1).[1,2] α-synuclein is a protein involved in controlling neurotransmitter release, hence aggregation within DA neurons inhibits dopamine transmission. Normally, DA neurons are responsible for transporting dopamine, an important neurotransmitter. Together, DA neurons control motor and behavioral processes. In particular, within the CNS, DA neurons provide specific membrane receptors for dopamine to bind onto which is part of further extensive interactions controlling locomotion, emotions, learning, cognition and memory.[3] Degeneration of these neurons in the SNpc, damages the basal ganglia's motor control network (brainstem), causing typical motor symptoms in PD: bradykinesia (slowness), tremors, postural instability and difficulty in speech and movement.[4] PD is, thus, categorized as a central nervous system disorder.

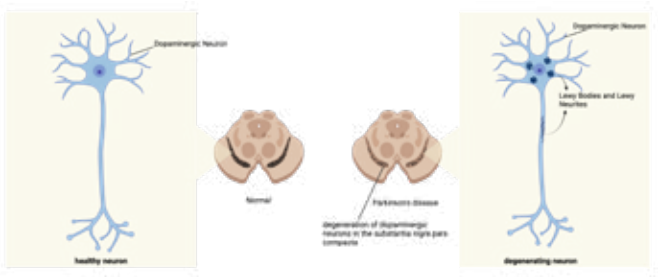

Figure 1: The normal versus the PD substantia nigra is illustrated above. In the normal brain, dopaminergic neurons can interact properly with dopamine transport in the CNS. However, in the PD brain, dopaminergic neurons degenerate, causing dysfunction in the neuron and CNS motor pathways. Lewy bodies and neuritis consist mainly of ambiguous filamentous material, with some suggesting aggregated α-synuclein as a component).[5] They are pathological hallmarks of neuron degeneration. Created with BioRender.com. Abbreviations: PD = Parkinson's Disease; CNS = central nervous system.

Due to the seemingly incurable nature of Parkinson's Disease (PD),[1] the development of regenerative medicine has been gaining traction in recent years, focusing on the disease's root cause – the degeneration of dopaminergic (DA) neurons in the substantia nigra (Figure 2). While results are hopeful, several challenges are also presented. Experiments show that cellular reprogramming is viable *in vitro* with artificially optimized conditions, however, the switch-ability of other cell types remains ambiguous. The efficacy of this procedure in different biological contexts, and the ability of TFs to share the ability to induce the desired cell fate switch *in vitro* for other cell types is unknown. Additional problems arise when trying to apply this to CNS disorders, like PD and includes: the switching of which specific cell types, the potentially harmful consequences of gaining neurons at the expense of some nonneuronal cells, the ability of induced neurons to reinnervate back into intrinsic neural circuitry (Figure 3). Specifically, research should prioritize the maintenance of long term viability of grafted neurons, limiting chances of tumorigenesis and immune rejection (and delivering vectors as well).[6]

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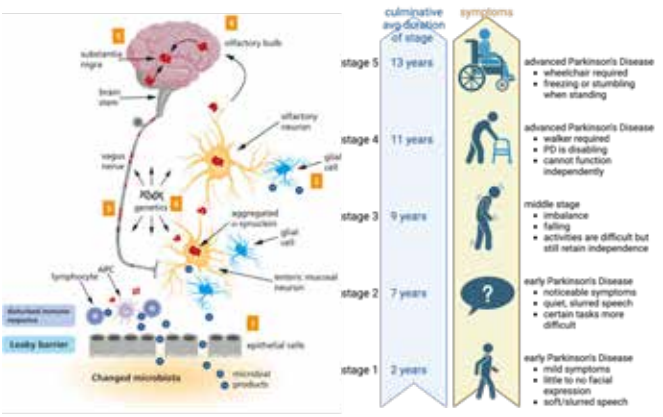

Figure 2: Modified from Rietdijkc C.D.;[7] information from Geest. V. J;[8]Braak's hypothesis and the corresponding symptoms of each stage are illustrated above. Microbial products can aggregate α-synuclein in olfactory and enteric neurons (1-2). Aggregated α-synuclein grows to the central nervous system via the olfactory bulb and vagus nerve (3-4). Eventually, aggregated α-synuclein reaches the substantia nigra (5). Although genetic factors are thought to play a part in Parkinson's disease, the exact mechanism remains unknown (6).[7] Created with BioRender.com.

This review provides a synthesized overview of current research from the past decade of in situ neuronal reprogramming – the most promising strategy for regenerative therapy thus far – and its applicability in PD. The key question for this regenerative therapy is whether degenerated nerve cells in the SNpc can be repaired, given the complex, gradually developed CNS circuitry, which may be irreparable in adulthood. To analyze this question, the review focuses on PD pathology, current therapies and their limitations, and the mechanisms of in situ cellular reprogramming with a focus on astrocytes – the leading cell type to be switched for PD therapy. To explain the complex process, the review will touch on the fundamental process and issues of adult neurogenesis. Finally, current progress and the translatability of *in situ* cellular reprogramming are discussed.

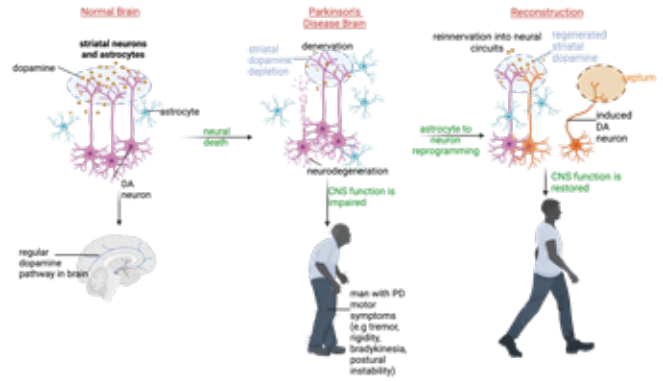

Figure 3: The illustration shows the recovery of the nigrostriatal pathway, crucial for induced neuronal activity in Parkinson's disease. Motor symptoms result from striatal denervation, necessitating the reconstruction of circuits through the generation of dopaminergic neurons and precise reinnervation of induced neurons. Striatal dopamine depletion contributes to Parkinson's symptoms (middle panel), while reprogramming neurons, as depicted in the right panel, holds theoretical promise for CNS function restoration and symptom reversal. It's worth noting that ambiguous septum-targeted axons may pose potential side effects.[9] Created with BioRender.com.

Parkinson's Disease Pathogenesis:

PD's pathological hallmark, DA neuron degeneration, can overshadow the fact that PD has a more complicated pathology that extends beyond the SNpc. Braak *et al.* hypothesize that PD is a six-stage process of neurodegeneration: (stages 1 and 2) the degeneration begins in the lower brainstem and olfactory system. During this stage, α-synuclein starts to aggregate and starts to out-populate globular Lewy bodies. The degeneration spreads up the brainstem. This causes the nonmotor symptoms of PD. (Stages 3 and 4) Neurodegeneration reaches the substantia nigra via the olfactory bulb and the vagus nerve, resulting in PD's typical motor symptoms. Severe DA neuron loss. The Lewy body α-synuclein aggregation starts to form in the SNpc. Lewy bodies continue proliferating in the brain stem affected by stages 1 and 2. (Stages 5 and 6) Neurodegeneration reaches the neocortex, impacting motor and sensory regions in the brain. This marks the most severe symptoms of PD, including Lewy body-induced dementia.[10] See Figure 4 and Figure 2 for a visual schematic. While this hypothesis proposed by Braak and his partners has yet to be fully corroborated,[11] it sheds light on complexity and progression of neurodegeneration beyond the SNpc and its pathology, impacting various brain regions.

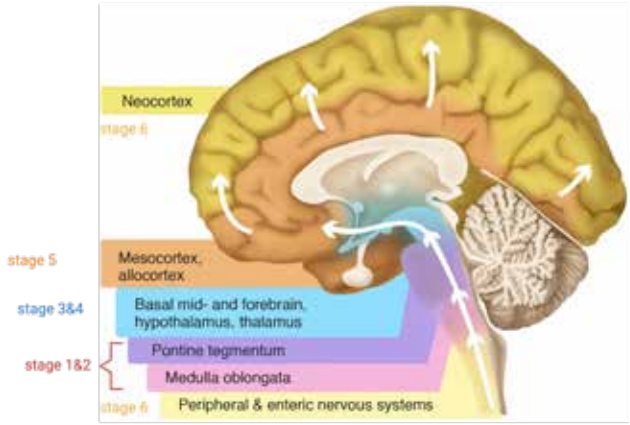

Figure 4: Modified from Visanji. N.[12] The path of neurodegeneration of Parkinson's disease in the human brain. Created with BioRender.com.

Current treatments for Parkinson's Disease and needs:

Currently, there are no cures for DA neuronal loss in PD, only symptom alleviation as listed in the previous section. This section will list the most popular therapies and their respective limitations.

i. Levodopa:

Levodopa (L-DOPA) is a powerful prescription medication that targets DA loss in PD by acting as a precursor to dopamine. One popular form, Sinemet, combines L-DOPA with carbidopa to increase bioavailability and enhance central dopamine production[13] by preventing unnecessary peripheral conversion of L-DOPA, improving the drug's efficiency. This treatment option (Sinemet) could be paired with DA agonists (or neurotransmitter "imposters") like ropinirole and pramipexole, though they can work alone as well.[13] Other medications such as Catechol-O-methyltransferase (COMT),

also be used alongside L-DOPA to inhibit the degradation of L-DOPA and DA. It thereby enhances carbidopa/L-DOPA activity; however, it also exacerbates side effects.[10]

Debilitating side effects, unfortunately, accompany L-DOPA therapy. The most prominent of them include dyskinesias induced by drugs, a disorder where movement becomes involuntary and irregular.[4] However, this risk is reduced when combated with DA agonists like ropinirole and pramipexole in the early stages of PD. This is a short-term treatment, however, since the efficacy of this treatment diminishes as the disease progresses and often requires more L-DOPA supplementation. Naturally, this increases the risk of dyskinesias for PD patients taking this medication.[1] As such, levodopa is not effective alone.

ii. Anticholinergic drugs:

PD's motor symptoms, such as rigidity, dystonia (involuntary contractions of muscles), and tremors, can be reduced using anticholinergic drugs. Some examples include trihexyphenidyl and benztropine. However, these are ineffective treatment options for bradykinesia (slow movement).[1] Side effects associated include increased heart rate, decreased urination, decreased gastrointestinal efficiency, and blurred vision, which can be detrimental in older patients.[14] As expected, nonmotor symptoms such as depression can be treated with the same therapies as non-PD patients. Conventional treatment options are as listed: "antidepressants, serotonin-noradrenaline reuptake inhibitors, serotonin reuptake inhibitors, or cognitive behavioral therapy," as outlined by Wei and Shetty.[1,10] Since non-motor symptoms are outside the scope of PD-specific drugs, their therapeutic limitations will not be focused on in this review.

In general, drug therapies for PD are reaching a therapeutic 'dead-end' by attempting to maintain a delicate balance of disease symptom control and minimizing drug side effects. This often requires the addition and subtraction of several drugs. The addition of anticholinergic drugs and inhibitors (listed above) is outweighed by the loss of DA neurons as PD progresses, necessitating L-DOPA despite the increased risk of dyskinesia, amongst other side effects.[10] Ultimately, drug therapy can be an effective treatment for symptomatic relief; however, as this does not target the neurodegeneration at the root of PD symptoms, it is limited in its long-term effects.

iii. Surgical therapy

Other therapies include surgical methods, like deep brain stimulation (DBS). This therapy targets different brain regions via electrodes for various therapeutic purposes. For example, targeting the mostly cholinergic neurons of the pedunculopontine nucleus via low-frequency stimulation is used to improve gait impairment in PD. The motor symptoms of PD can be further treated by delivering high-frequency DBS to the subthalamic nuclei (STN). It has been observed that this also hinders PD's neurodegeneration progression, which Benabid et al. notes, is likely due to inhibition of STN's output of neurons.[10,15] Alternative treatments that target tremors can also be used, including DBS of prelemniscal radiations (fibers located within the posterior subthalamus) and caudal zona incerta.[15] On its own, the surgery's success in symptom control stands at 75%;[16] however, DBS does not permanently stop PD disease progression.

iv. Summary:

Ultimately, current therapies are lacking in terms of treating the root of PD's neurodegeneration in the SNpc. Symptomatic treatment does not seem to have long-term inhibition of PD progression which is notorious for becoming worse with age. PD may even reach a point where medications and other therapies will have adverse reactions or have no effect at all.[17] Therefore, alternative therapy that addresses the issues of the current treatments listed, mainly immune rejection and DA neuron degeneration in PD, is required to improve these patients' quality of life.

In situ cellular reprogramming: how does it work? :

A potential therapy that has gained increasing traction in recent years targets this neurodegeneration, called *in situ* reprogramming. This involves either direct or indirect reprogramming of endogenous nonneuronal cells into DA neurons in the SNpc. This section will dissect the mechanisms of in situ cellular reprogramming by first outlining the fundamental process of adult neurogenesis which reprogramming exploits. Subsequently, focusing on the leading reprogrammable glial cell, astrocytes, I look closely at two of many reprogramming pathways: TFs and miRNAs (Figure 5).

i. Adult neurogenesis :

In situ direct cellular reprogramming relies on adult neurogenesis, the postnatal regeneration of neurons from type 1 neural stem cells (NSCs) such as radial glia. Glia cells, like astrocytes, emerge later in development (Figure 6).[10] It is well known that adult neurogenesis has an age-dependent decline in humans. However, this concept central to cellular reprogramming is the potential for the existence of neural stem cells (progenitors) to be preserved in adulthood. Aging likely reduces the population of these special cell types, or the latency of these cells could increase with age, as there must be a starting population for neuronal rejuvenation in the adult brain.[18] *In situ* neuronal reprogramming is the novo technique for 'waking up' these latent neuronal stem cells to produce functional neurons in diseased brains like PD. Interestingly, the PD-affected-adult brain regions, such as the SNpc and the striatum, have little observed neurogenesis activity (Figure 6). As a result, glial cell reprogramming into DA neurons has become a hot topic in PD models and could hold the key to curing neurodegeneration.

Adult neurogenesis in humans is still a topic of intensive debate. An earlier study by Spalding *et al*. hypothesized that roughly hundreds of neurons are being produced daily from NSCs and can incorporate themselves into the human brain's DG network – essential for neuron function (Figure 4).[19] In contrast, two recent publications have reached a different conclusion regarding hippocampal neurogenesis. One paper concluded that neurogenesis stopped after puberty[20] and another observed that neurogenesis continued despite age.[21] The main cause of conflict seems to lie in quantitative differences in the adult human brain, likely due to alternative techniques and biomarkers used.[22,23] It is safe to conclude from these two studies, however, that while neurons can be generated in the

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early postnatal human brain, the extent of this remains ambiguous.

Figure 5: The mechanisms of *in situ* cellular reprogramming simplified. Created with BioRender.com.

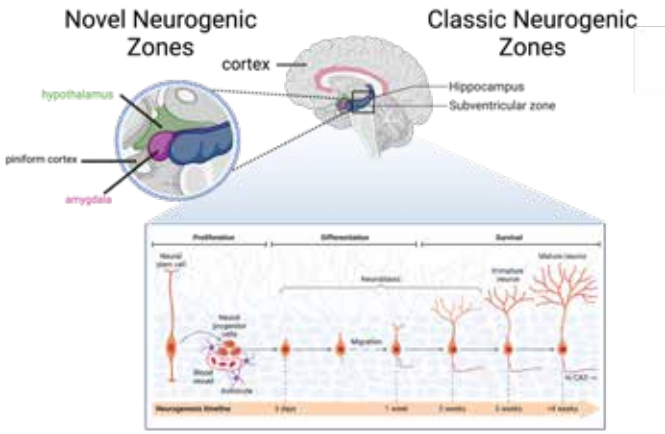

Figure 6: Illustration of "novel" and standard adult neurogenic zones, including their main cellular stages. The "novel" adult neurogenic zones are depicted on the left: the "prefrontal cortex, hypothalamus, striatum, amygdala, and piriform cortex".[24] Similarly, the primary adult neurogenic zones are depicted on the right-hand side: the hippocampus and the subventricular zone (SVZ), which is the source of progenitor cells. Created with BioRender.com.

ii. The key players: astrocytes and associated Transcription factors, microRNAs:

A popular example of reprogrammable glia cells is astrocytes. Astrocytes are specialized cells found throughout the CNS and are considered nonneuronal. Their main function is to maintain homeostasis within the CNS and assist in integral neuronal activities. Recent studies have unveiled a key possibility for astrocytes, the brain's most abundant nonneuronal cell type, to turn back into neuronal progenitors in response to brain injury.[10,25] This is likely due to their highly heterogeneous nature in various brain regions,[9,26,27] or in other words, the cell type's origins, morphology, and function are varied. This heterogeneity is important in neuronal reprogramming as it is likely an important contributor to astrocyte specificity and efficiency; these characteristics make astrocytes an ideal target especially when programming for specific disease settings such as PD.[9,28-30] Hence, for the purpose of this review, I will focus on two in situ astrocyte reprogramming mechanisms as a therapeutic strategy for PD: transcription factors and miRNAs.

a. Transcription factors:

Following the success of reprogramming astrocytes to neuronal progenitors[31] and iPSCs,[32] research has focused on using lineage-specific transcription factors (TFs) to engineer cell fate, coined as the "addition strategy" by Qian & Fu.[9] Clinical trials of this technique are currently limited due to insufficient understanding of the mechanisms needed to accurately predict cell type switching and the required TFs. However, analysis of networks[33] that regulate genes or dissect single-cell sequencing data is being implemented to uncover effective reprogramming pathways.[34]

Lentiviral transduction efficiently induces pluripotency, making it a promising tool for cellular reprogramming via the "addition strategy". It can transduce cells at all stages, not just during mitosis, making it ideal for reprogramming differentiated cells (Figure 7). For example, Niu *et al*. successfully converted mouse astrocytes into mature neurons in situ, capable of circuitry reinnervation, using Sox-2 via a lentiviral delivery system.[35] Similarly, adeno-associated virus (AAV) encoding Ascl1[36] and overexpression of Zfp521[37] have reprogrammed astrocytes into neurons, astrocytes, and oligodendrocytes. The neurons induced by Zfp521 displayed glutamatergic and GABA-ergic markers, mirroring the nigral striatum environment required for healthy DA neuronal regulation, showing great potential as a PD therapy method. Overall, lentiviral transduction is currently advanced enough to require only one TF's overexpression to directly reprogram astrocytes into neurons in situ in addition to observed successful induced neuron reinnervation. This could open a more efficient pathway for cellular reprogramming in neurodegeneration treatments.

Qian & Fu also discuss the "subtraction strategy", whereby proteins are inhibited.[9] Neuron generation can be enhanced by removing the key negative regulator. REST is a key negative regulator involved in maintaining neuronal progenitors in the brain and seems to be key for lengthening the lifespan of mature neurons in the CNS,[38] making its inhibition unsuitable for reprogramming. However, the subtraction strategy could have a regulatory role in cellular reprogramming through targeting other master negative regulators. A recently discovered example is *Polypyrimidine* tract-binding protein 1 (PTBP1). It is ubiquitously expressed in most cell lineages besides mature neurons, making it a promising target for reprogramming regulation. PTB protein represses splicing of neuron-specific coding regions whereas its homolog nPTB is upregulated during neuronal differentiation.[10] PTBP1 expression gradually decreases during neurogenesis.[39,40] Notably, the reduction of PTBP1 levels was observed to reprogram multiple cell types into the neuronal lineage *in vitro*,[41] including DA neurons.[42] More uniquely, the astrocyte-derived DA neurons generated in the substantia nigra were discovered to be able to reinnervate damaged circuits and, thereby, restore striatal dopamine in the brain region (Figure 4). PTBP2 is briefly activated, key for the induced neurons to mature. These studies open another promising programming pathway for PD using PTBP1. Further efforts to refine this technique and fully exploit its relationship to PTBP2 may be useful in future therapeutic research.

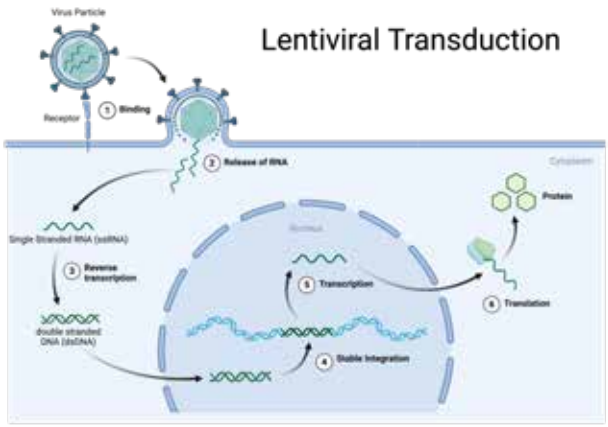

Figure 7: A more specific method illustration of reprogramming using lentiviral vectors, known generally as lentiviral transduction. Lentiviruses, a subclass of retroviruses, can permanently integrate into the host cell's genome. After the virus enters the cell, reverse transcriptase transcribes the viral RNA into double-stranded DNA that enters the nucleus. Finally, the transgene is integrated into the host genome using the lentiviral integrase enzyme. While using lentiviral transduction, users must consider the effects of genomic integration.[43] Created with BioRender.com.

b. microRNAs:

On the other hand, miRNAs can differentiate astrocytes into mature neurons by regulating gene expression at a post-transcriptional level. Two commonly used miRNAs are miR-124 and miR-9. miR-124 is expressed in differentiation and mature neurons while the latter is expressed in neuronal precursors.[44] Part of their use in neuronal reprogramming is its inhibition of Class IIa histone deacetylase (HDAC5) expression – a key inhibitor of neuronal differentiation, specifically in the CNS.[10,45]

Experimental research has varied in success. Yoo *et al.* showed that human neonatal foreskin fibroblasts can be reprogrammed into neurons via the expression of miR-9 and miR-124. Using a lentiviral vector that encoded the corresponding miRNAs' precursors, they could transduce fibroblasts into neuron-like cells.[46] It is, however, important to note that the switch rate was less than 5% with only the miRNAs. This rate increased to approximately 50% when combined with a neurogenic TF, suggesting that collaborative reprogramming techniques are ideal for future therapeutic development. In addition, miR-124 and miR-9 target BAF complex subunits, involved in neural development,[47] and other genes like REST and PTBP-1 to induce neurogenic differentiation.[46] This suggests miRNAs can enhance TF-induced reprogramming strategies. BAF complexes are known to regulate epigenetic modulations across multiple regions of the genome in ESCs,[47] meaning it may be possible for miRNAs to be able to reprogram cells by epigenetic intervention. Alternatively, exogenous miRNAs can also directly reprogram astrocytes into neurons.[47] This has been demonstrated in the striatum with induced adult mouse and human astrocytes. A lentiviral vector encoding miR-302/367 cluster was used to directly reprogram the astrocytes into neuroblasts. Direct reprogramming reduces the risk of teratoma production, so this method is therapeutically ideal.

Alternatively, modifying miRNA targets like PTB can promote neuronal expression. An example is suppressing PTB with miR-124 which facilitates direct reprogramming of fibroblasts into neurons.[48] However, the REST complex inhibits many neuronal genes in nonneuronal cells, like miR-124, which could inhibit reprogramming. At the same time, miR-124 suppresses REST, SCP1, and co-REST, which is involved in a regulatory loop for neuronal expression.[48] As PTB reduces the suppressing actions of miR-124, PTB is an integral target that miR-124 should suppress to increase neuronal gene expression,[48] opening a new avenue of neuronal reprogramming. Hence, as outlined in the previous subsection, further research into the PTB/nPTB loop with TFs and miRNAs could be a potential alternative method for DA neuron regeneration in PD therapies.

Reactive gliosis and injury-induced astrocyte reprogramming:

Injury-induced reprogramming is another potential method for astrocyte reprogramming, though its therapeutic effect in humans remains unclear. In response to CNS injury, astrocytes become activated, and the brain area becomes inflamed, a process called reactive gliosis or glial scar formation. Magnusson *et al.*'s work documents a modest amount of *in vitro* astrocyte differentiation into neurons in response to CNS injury,[25] reflecting a new approach to driving dedifferentiated astrocytes into a neuronal cell fate. However, glial scar formation can prevent neuronal regrowth, posing a challenge for PD therapy that requires astrocyte conversion into DA neurons.

Interestingly, while neuronal reprogramming can be engineered without injury in mice,[42] some suggest that an 'injury site' may have specific benefits. The injury site would be manufactured using the mechanical impact of stereotactic injection. Regarding possible PD therapy, injury-induced reprogramming could provide enough local cues for a global positioning system (GPS).[9,36] This means reprogramming agents can have a more specific target in brain regions, such as SNpc and striatum, requiring repair. This is particularly beneficial in PD, where damaged neurons are not well-defined in the corresponding brain regions. For example, although DA neurons are strongly linked to motor symptoms in PD, nonmotor symptoms caused by dysfunctional neurons remain unclear.[48,49]

In this view, it is interesting to consider an alternative use of reactive gliosis for cellular reprogramming. There is potential to use reactive gliosis to potentiate cell fate switch via epigenetic reprogramming – whereby the genome is more exposed to reprogramming factors. The possibility of this emerged in Brulet *et al.*'s study, demonstrating that when reactive gliosis was present, the results demonstrated higher conversion efficiency in NeuroD1- mediated neuronal programming in the striatum.[51,52] The study further implies that reactive gliosis itself is not a prerequisite for neuronal reprogramming, suggesting that a more efficient reprogramming approach exists.

Additionally, reactive gliosis involves two types of astrocytes: A1, which is harmful, and A2, which is helpful for brain repair. A1 astrocytes are linked to the pathology of PD.[9,53] A2 astrocytes tend to proliferate at a higher rate than A1 astrocytes. In light of these discoveries, perhaps it is possible for future PD therapies to convert A1 astrocytes into DA neurons, thereby minimizing their symptomatic contribution. Alternatively,

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could the proliferation rate of A2 astrocytes be exploited by reprogramming them into DA neurons?

Ultimately, exploring reactive gliosis and questions posited above in humans may be better compared by conducting more clinical trials in non-human primates (NHPs) before considering this method as a possible PD treatment.

Progress in direct cellular reprogramming in situ for PD:

This section details the significant advances in direct neuronal reprogramming for PD brain repair from 2017 onwards. The first milestone was demonstrated in 2017 by di Val Cervo *et al.* (Table 1), where they converted astrocytes to DA neurons in a PD-specific disease setting. Three transcription factors were used; NeuroD1, Ascl1 and Lmx1A, and the microRNA miR218 (coined NeAL218). In the mouse brain model, induced DA neurons were produced by reprogramming human astrocytes *in vitro* and mouse astrocytes *in vivo*.[54] The PD mouse brain model showed successful reprogramming of adult striatal astrocytes into induced DA neurons using only NeAL218, which improved motor symptoms such as gait. These observations posited the potential for delivering genes rather than cells in PD clinical therapy.[54] Following this, in 2020, H Qian *et al.* (Table 1) made another breakthrough in direct neuronal reprogramming by rebuilding the nigrostriatal pathway in PD by inhibiting expression of the RNA-binding protein PTB. Using a chemically induced model of PD, they demonstrate the reprogramming of midbrain astrocytes into DA neurons whose axons can, uniquely, rebuild the nigro-striatal circuit. Induced neurons can, thereby, reinnervate into existing circuits. This is followed by the elevation of DA levels and improved motor impairment. The applicability of this research for therapeutic purposes in PD is discussed in the next section.

Table 1: Clinical progress in astrocyte to-neuron reprogramming for PD so far; modified from Qian and Fu.[9]

Year	Factor(s)	Species	Region(s)	Circuit Reconstruction	Behavioral Benefits	References
2017	NeuroD1, Ascl1, Lmx1a, miR218	mouse	Cerebral cortex, striatum	Not Tested	yes	[54]
2020	PTBP1 depletion	mouse	striatum	yes	yes	[55]
2020	PTBP1 depletion	mouse	The midbrain, cerebral cortex, striatum	yes	yes	[42]

Discussion

This section will discuss some of the afore-mentioned challenges, research, and future directions that should guide future efforts.

Challenges of Indirect reprogramming involve the risk of teratoma formation[56] associated with the intermediate iPSC stage. By avoiding this stage, direct reprogramming resolves this problem. Another issue is the immune rejection risk in the host from the viral vectors used in direct and indirect reprogramming. Studies show immune reactions to the AAV vector, commonly used for supplementing TFs, posing therapeutic problems. Transient immunosuppression like methylprednisolone could minimize this risk. Other methods to combat the immune reaction to AAV vector antigens include increasing AAV type 2 (AAV2) activation efficiency by inhibiting proteosome-mediated degradation through epidermal growth factor receptor protein tyrosine kinase inhibition.[57] While improving viral vector delivery is important, using non-immunogenic methods may be more effective. For example, small molecules can safely deliver TFs or miRNAs via hydrogel technology and have successfully reprogrammed mouse and human fibroblasts into DA neurons.[58] Furthermore, small molecules are less costly than viral vectors. Their pre-existing protocols could easily be standardized as well, which may be useful for large-scale therapy production.[10]

As aforementioned, future research should prioritize enhancing reprogramming efficiency and induced neuronal survivability. In relation to this, newly reprogrammed DA neuron maturation *in situ*, like the substantia nigra, should be thoroughly analyzed before further clinical trials. It has been demonstrated that different brain regions and injury environments may affect reprogramming success and the survival of induced neurons.[59] Notably, growth factors and Neurog2 in the striatum show varying efficacy and maturation compared to the neocortex, with the striatum having fewer neuronal conversions. Neurog2 alone boosts early neuron proliferation in the neocortex, but this diminishes over time. Injury in these regions increases neuron proliferation and maturation.[59] Hence, while brain regions play a part in reprogramming efficiency, induced neurons maturation and subsequent lifespan are critical challenges that should be resolved before further clinical trial research. Otherwise, clinical trials will yield limited progress.

To increase reprogramming efficacy *in situ*, reprogramming success rates should be mapped during PD degeneration stages providing the optimal timeframe[9] for this therapy. Another challenge is the reinnervation of neural circuitry. To regenerate neuronal networks, induced neurons must deliver axons to specific efferent sites and form correct synapses with the desired neurons. This will ultimately liken the odds of successful recovery after CNS injury of neurodegeneration.[59] It is critical to note that structural differences between rodent and primate brains could hinder the regeneration of neural pathways in nonhuman primates (NHPs) and, even more significantly, humans.[60] For example, the human brain is around 3800 times larger than the mouse brain, contributing to the hurdle of long-distance neuronal targeting to reconstruct dysfunctional networks.[9] Hence, the induced neurons' ability to reconstruct neuronal networks need to be further explored in NHPs rather than rodent brains for therapeutic contexts.

Significant advancements in nigrostriatal pathway reconstruction have made it possible to produce fully functional DA neuron replacements for PD therapy. However, it has currently only been demonstrated in a mouse model of the PD brain.[42] Future research should also focus on improving the modeling technologies to incorporate the characteristic, age-dependent degeneration of NDs to allow for more accurate predictions of reprogramming effects in clinical trials. Failure to form functional neural circuits or misprediction of side effects could produce abnormal connections with adjacent neurons and subsequent neuronal hyperexcitability in the reprogrammed regions – increasing the risk of seizures.[61] Additionally, the side

effects of regenerating neurons at the expense of nonneuronal cell types like astrocytes could be better predicted.

Conclusion

The review highlights the potential of *in situ* cellular reprogramming as a therapeutic strategy for Parkinson's Disease (PD) by regenerating dopaminergic (DA) neurons, unlike current treatments that only alleviate symptoms. It discusses the mechanisms of reprogramming through two primary methods: transcription factors (TFs) and microRNAs (miRNAs). Astrocytes, a promising non-neuronal cell type for reprogramming due to their abundance and similarity to radial glia, are emphasized. The review also explores reactive gliosis and injury-induced reprogramming as an alternative method.

Key challenges for cellular reprogramming include improving efficiency, ensuring neuronal survival, and addressing limited neurodegeneration models. Future research should identify more non-neuronal cell types for reprogramming and prioritize long-term studies on the maturation and longevity of reprogrammed DA neurons. Enhancing modeling technologies to better simulate the age-dependent neurodegeneration in PD affected brains and conducting more clinical trials in non-human primates (NHPs) are crucial for more accurate predictions of therapeutic effects in humans.

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Author

Katherine Lau recently graduated in 2023 from Abbotsleigh in Sydney, Australia. She is interested in pursuing undergraduate medicine in the UK. Alongside science, she is interested in many different art forms such as drag and animation.

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