Applications and Challenges of Neural Stem Cell Therapy

John Brewster

A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2022

This Senior Honors Thesis is accepted in partial
fulfillment of the requirements for graduation from the
Honors Program of Liberty University.

Dr. Kimberly A.P. Mitchell, Ph.D.
Thesis Chair

Dr. Paul Sattler, Ph.D.
Committee Member

Dr. Marilyn Peyton, Ph.D.
Honors Assistant Director

April 20th, 2022

Abstract

In response to the many neurological disorders that plague humanity, no treatment shows more promise than stem cell therapy. By using these special cells to regrow damaged neurons and combat sources of disease in affected patients, researchers hope to treat neurological disorders of all kinds. While great strides have been made in laboratory settings, the widespread use of stem cells to treat neurological disorders in humans is still a distant goal. Recent advancements have been made in the area of neural stem cell therapy, but complications arise when using this method to treat neurological disorders.

Applications and Challenges of Neural Stem Cell Therapy

Stem cells possess the remarkable ability to develop into many different types of cells and are the foundation of every tissue and organ system of the human body. While all stem cells can self-renew and differentiate, different types of stem cells have a range of abilities. For example, embryonic stem cells are those that have been extracted from the blastocyst, a mass of cells that forms days after a human egg cell has been fertilized by a sperm. These cells are valuable for their pluripotent ability, or the ability to transform into every cell in the body, except for placental or umbilical cord cells. Tissue-specific stem cells, on the other hand, are adult stem cells that are housed in a specific organ and can transform into any cell of the same organ. While using these cells to combat disease shows enormous potential, the many setbacks that have occurred when transplanting stem cells show that advancements need to be made for widespread use of this method to be possible (Alison et al., 2002).

One of the most promising applications of stem cell therapy lies in the prospect of using these specialized cells to treat neurological disorders. Neurological disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, cerebrovascular diseases, and many others arise when some factor induces the destruction of nervous tissue. When a neurological disorder causes damage to the neurons of these individuals, long-term and potentially permanent effects can arise due to the inability of most neurons to regenerate. This is due to the amitotic nature of neurons, meaning that the DNA copying of these cells is impeded by a lack of centrioles, making them unable to enter mitosis. While this might seem like a flaw, the specialized functions that neurons perform to transfer signals throughout the body require neurons to establish widespread interneuron connections (Frotscher, 1992). The addition of new neurons by the replication process would harm these functions by taking energy away from

signal transduction and interfering with established neural connections. The goal of neural stem cell therapy is to combat damage caused to these neurons by inducing the differentiation of new neurons.

While the inability of neurons to reproduce is constant throughout much of the body, there is evidence that neural reproduction (neurogenesis) occurs in two main regions of the body: the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (Haas et al., 2005). In addition to this finding, it has also been found that neural stem cells (NSCs) exist in the striatum, spinal cord, and neocortex of the central nervous system. The NSCs in these areas persist throughout the lifetime of humans and are exogenously modulated by external signals. When signaled by factors such as an enriched environment, physical activity, or stress, neurogenesis begins.

Therapeutic Process

Neural stem cell therapy begins with the culturing and replication of NSCs in laboratory conditions. While there are multiple ways to obtain NSCs, researchers need to be aware of the limitations of each method. The first way that these cells can be obtained is by extracting them directly from neural tissues such as the subgerminal zone in adults or the neuroectoderm in fetuses (Boese et al., 2018). Once obtained, the stem cells are grown on media, and growth factors are added to increase expansion. Another method used to obtain neural stem cells is to derive them from embryonic stem cells in vivo. This technique, however, is limited by the vast amount of manipulation that is required to cause the embryonic stem cells to differentiate into NSCs instead of other cell types. To maximize neural stem cell yield, certain compounds need to be introduced into the embryonic stem cell culture to inhibit differentiation into other cell types.

In addition to the previous two techniques, there also exists a method for obtaining NSCs by reprogramming somatic cells to reproduce stem cells. iPSCs, which are human-induced pluripotent stem cells, have been created by using vectors to introduce new genes into somatic cells, causing them to reproduce stem cells instead of somatic cells. One benefit of using iPSCs to generate neural stem cells is there are fewer ethical concerns than traditional methods of deriving stem cells from human embryos. This is especially important due to the knowledge that such types of embryos are increasingly often showing developmental autonomy and even viability (Denker, 2021). However, when working with iPSCs, a limitation exists in having to navigate the lengthy procedure of taking already differentiated somatic cells and reprogramming them back into their normal state. Another method similar to this involves using defined growth factors to directly cause the differentiation of somatic cells into neural stem cells (Boese et al., 2018). This method shows much promise because, in addition to limiting ethical concerns regarding extracting fetal tissue and reducing the risk of immune system complications, the lengthy process of turning somatic cells into iPSCs is avoided.

Once NSCs have been obtained, they must be grafted into the target issue and successfully migrate to the site of neural damage for therapeutic effects to be performed. In many cases, it has been found that cells transplanted into the brain to treat injury form clusters near the site of injection and demonstrate a low migratory capacity (Ladewig et al., 2014). One hypothesis for this occurrence is that the level of maturity of the transplanted cells is important when hoping to achieve proper cellular migration. In a preclinical study, cortical neuroepithelial stem cells (cNESC) were derived from iPSCs and transplanted into a rat stroke model 7 days post-injury (Payne et al., 2018). Before transplantation, the cNESC cells were arrested in three different stages of differentiation: early-, mid-, and late-stages. At the end of the study, it was

found that a higher number of graft cells were observed to survive in rats receiving the early- and mid-stage differentiated stem cells. This shows that the level of stem cell maturity should be considered when seeking to establish cell lines for transplantation.

Chemoattraction is another factor to consider when investigating NSC migration. Many studies have demonstrated the remarkable chemoreceptive abilities of NSCs. When grafted into a tissue of interest, these cells will naturally migrate towards areas of injury. This is due to chemokine receptors that allow NSCs to recognize areas of increased pro-inflammatory chemokine expression (Boese et al., 2018). While this can be a helpful property of NSCs, it can also work to counter migration. It has been found that factors such as fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF), which are naturally expressed by neural progenitor cells, can act as chemoattractants for NSCs, pulling them away from injury sites (Ladewig et al., 2014). To counter this, researchers have used FGF2 and VEGF inhibitors to pre-treat soon-to-be grafted cells. It was found that by using this method, more transplanted cells underwent proper migration and less densely packed engraftment was observed one-week post-transplantation. Once neural stem cells arrive at an injury site, they can begin to reproduce neurons to make up for those that have been damaged or destroyed.

Neural stem cell therapy, being a relatively new therapeutic technique, has shown both extreme promise and various complications. Some of the successes of neural stem cell therapy have come from preclinical studies on animal models, where results suggest that behavioral recovery after neurological damage is the result of new neural connections being established between the brain and grafted NSCs (Cardoso et al., 2018). These new neural connections support the conclusion that the implanted neural stem cells were able to overcome compatibility obstacles with the animal host and effectively treat disease. Additionally, clinical trials on

patients with Parkinson's disease have been conducted where grafted fetal dopaminergic neurons were able to improve motor function (Lindvall et al., 1992). While these results are encouraging, complications also exist in using neural stem cell therapy to treat neurological disorders. For example, in clinical trials involving the implantation of NSCs, adverse post-surgery effects such as immune reactions and tumor growth have arisen, and the survivability of grafted neural stem cells has been shown to vary widely between trials (Li et al., 2007). For neural stem cell therapy to be ready for wide employment as the premier method for neurological disease treatment, researchers need to uncover solutions to these complex obstacles.

Immune Response to Transplantation

Much of the difficulties in transplanting NSCs come from the body's natural reaction to foreign material, which is to generate an immune response. This response, although quantifiable, can vary based on the type of transplant being conducted. An autograph transplant, for example, involves grafting self-tissue into another area of the same individual (Punt et al., 2019). Autograft transplants have a high likelihood of avoiding the generation of a severe immune response because the transplanted tissue is derived from the same individual it will be transplanted into and hence will not contain "anti-self" markers in the graft tissue. Another type of transplant, an isograft transplant, also avoids generating a harmful immune response by grafting tissue that comes from genetically identical individuals such as identical twins. Since these individuals contain the same genetic code, they also contain most of the same cell markers. Unfortunately, most organ transplants today require xenograft or allograft transplants, which are when an organ or tissue is transplanted between different species or separate individuals of the same species, respectively. In both of these types of transplants, tissue rejection is common and follows a predictable clinical course

In the first stage of graft rejection, called hyperacute rejection by preexisting antibody, rejection occurs before graft tissue can revascularize, due to complement pathways being activated by antibodies (Salado-Manzano et al., 2020). These pathways target graft tissue shortly after it is transplanted into a host. Following this stage, acute rejection occurs via mediation by T-cell responses. In this stage, alloantigens expressed on cells of the foreign graft are recognized by $CD4^+$ and $CD8^+$ T cells and proliferate in response (Punt et al., 2019). Usually, acute rejection occurs a week or two after transplantation has occurred. Chronic phase rejection, the last stage of transplant rejection, occurs due to both cell-mediated and humoral responses to graft tissue. Unfortunately, this phase of rejection is very dangerous because it can occur months or even years after acute rejection reactions to transplanted tissue have subsided. Currently, the survival rates of individuals receiving grafted tissues and organ transplants are the highest in history, but, even so, clinical research is currently being conducted to attempt to discover allograft transplantation techniques that would avoid generating harmful immune responses and risking tissue rejection altogether.

Avoiding Harmful Inflammation

After neurological disorders such as stroke take place, it is common for harmful inflammation to be generated, causing secondary damage to both the central and peripheral nervous systems. This inflammation is caused by the actions of the body's innate and adaptive inflammatory mechanisms (Lee et al., 2008). This process begins when neutrophils and macrophages initiate the infiltration of microglia into the brain. Following this cell migration, the site of neurological damage experiences widespread activation of inflammatory cytokines and chemokines. Next, the major cytokines, nuclear factor-kappa B (NF-kB) and tumor necrosis factor-alpha (TNF-α) are activated by the spleen, generating a systemic inflammatory response.

Due to the unique ability of neural stem cells to facilitate the regeneration of damaged neurological tissue, its promise for treating common disorders such as stroke is unmatched. Recently, it has been found that NSCs are even more valuable in medicine due to their remarkable abilities to attenuate the production of a harmful inflammatory response. The method by which grafted neural stem cells accomplish inflammatory attenuation is known as the "bystander effect" (Lee et al., 2008). During this action, NSCs show chaperone-like roles to modulate their surrounding environment and use immune-like functions to induce the regulated cell death, or apoptosis, of encephalitogenic T-cells. Additionally, NSCs have also been shown to suppress the adaptive immune system, which leads to decreased peripheral inflammation generation.

Because one of the chief causes of transplant rejection is widespread inflammation, it is clear to see why it is so important that NSCs alleviate this concern. While most transplantation surgeries require dangerous immunosuppressive treatments before a procedure is viable, NSCs can create an environment safe enough for graft tissue to survive in a new host without the involvement of outside pharmaceuticals. In this way, continued research into advancing the natural anti-inflammatory and immunosuppressive abilities of NSCs may allow future patients with neurological disorders such as stroke, Parkinson's disease, or dementia to heal in a way that does not risk developing a serious or even life-threatening infection.

Genetic Modification

An additional strategy to improve the viability of neural stem cell transplantation is employing genetic modification to improve both the survival rate and therapeutic function of transplanted cells. Through gene editing, researchers have been able to insert exogenous genes into the NSC's genome, allowing much larger numbers of grafted cells to survive implantation.

While one of the most promising functions of NSCs is their natural immunosuppressant and anti-inflammatory abilities, which help to avoid the risk of generating rejection, there have been concerns in the medical community about whether genetic modification of NSCs will lead to the generation of an increased immune response. These concerns have mostly arisen from data on other genetically modified stem cells, such as mesenchymal stem cells (MSCs), which show harsh immunological reactions following genetic modification (Wei et al., 2021). However, if researchers can genetically modify NSCs while retaining their ability to thwart tissue rejection, a new avenue for treating neurological disorders could be opened.

In a recent study, NSCs were genetically modified with the magnetosome membrane-specific gene before transplantation into host tissue (Wei et al., 2021). As major histocompatibility complex (MHC) classes I and II play important roles in the regulation of immunological responses, the research focused on analyzing these complexes as well as modified NSCs, astrocytes, and microglia. While the concentrations of astrocytes, microglia, and MHC molecules were found to have increased immediately following transplantation, it was discovered that the concentrations of these cells and molecules were decreased overall after three weeks. Also, when these results were compared to studies with non-transgenetic NSCs, it was found that genetically modified NSCs were not responsible for the aggravation of immunological responses.

Immunosuppressive Therapy

Because the innate immunosuppressive abilities of NSCs, while remarkable, may not be fully able to limit harmful responses enough to avoid rejection, therapeutics may still need to be employed to mitigate risks. In a recent study on human embryonic stem cells (hESCs), tissue regeneration and recovery have been observed in animal models of Parkinson’s disease (Snow et

al., 2019). Since hESCs can differentiate into virtually any cell type in the body, it can be reasonably assumed that the neurological recovery observed in the previous study stemmed from the cells' differentiation into neural tissue. When evaluating the viability of using hESCs to treat immunocompetent versus immunosuppressant animal models (mice), it was discovered that the survival rate of the immunocompetent mice with grafted hESCs was far below that of the immunosuppressed mice containing grafted hESCs (Swijnenburg et al., 2008). This is due to cellular and humoral immune responses characterized by inflammatory cell infiltration and subsequent immune-mediated rejection of hESCs in immunocompetent animals. Drawn from the above study, it can be concluded that immunosuppressive techniques can lead to a decreased chance of transplant rejection, even in stem cells that are being used to treat neurological diseases.

Development of Lineage Control

One of the leading complications of neural stem cell therapy is the complexity of controlling NSC differentiation. If a solution is not found for this problem, NSC transplants may become widely unavailable for fear of creating large populations of unwanted cell types. One possible solution to this problem comes from the immobilization of growth factors and cytokines, molecules that can modify stem cell function (Li et al., 2014). For these growth factors to be successfully delivered to the area of interest, researchers have engineered scaffolds that provide a matrix for cell attachment, migration, and differentiation. In a recent study, researchers successfully used a modified chitosan hydrogel system to encourage neural stem cell differentiation with adsorbed and immobilized growth factors.

The chitosan scaffold that was employed in this experiment was found to successfully deliver growth factors to NSCs, and, as a result, allowed researchers to control the specific

lineages being created. For example, researchers delivered the growth factor interferon-γ (IFN-γ) to direct the NSC production of neuronal cell types, platelet-derived growth factor-AA (PDGF-AA) to direct the production of oligodendrocytes, and bone morphogenic protein-2 (BMP-2) to direct the production of astrocytes (Li et al., 2014). While these results were obtained from research involving cell cultures, they show much promise for application to human disease. If researchers can use a similar scaffold to the one used in this experiment to apply growth factors to the human spinal cord, for example, expectations could be made for guiding structures that would replicate the developmental formation of the CNS and replace damaged tissue. In this way, the applications for partnering this biotechnology with NSCs to treat sites of neurological damage are enormous.

Alzheimer's Disease

Of the many neurodegenerative disorders that plague humanity, Alzheimer's disease (AD) has recently become among the most prevalent. AD is characterized by progressive impairment of memory and other cognitive functions, and it is currently estimated that one in every ten people over the age of 65 in the United States is afflicted by this disease (Hebert et al., 2013). With the prevalence of this disease and the resulting toll it has on society increasing as the population of the United States ages, a considerable amount of research is currently being conducted to find new treatment methods for this currently incurable disease.

While the many factors that lead to the development of AD are complex, much evidence supports a few specific hypotheses relating to the onset of this disease. To start, the amyloid-cascade hypothesis has shown that the accumulation of amyloid-β in the parenchyma and cerebral blood vessels triggers many of the events leading to neurodegeneration (Hardy & Selkoe, 2002). When amyloid-β accumulates in these areas of the brain, it has been found that

the structural integrity of the blood-brain barrier, a brain structure that serves a critical function in brain homeostasis as well as the clearance of amyloid-β from the central nervous system, degrades significantly. Interestingly, it has also been found that in rare cases, individuals exhibiting homozygosity for genes controlling amyloid-β production have exhibited partial protection from neurodegeneration (Hampel et al., 2021). In these instances, the rare mutation being exhibited allows the individual to continue normal cognitive functioning, even in the presence of a severe number of amyloid-β plaques. However, the patients exhibiting these genes eventually developed dementia-like symptoms as a result of amyloid-β and neurofibrillary tangle accumulation. As a result of these discoveries, it has become essential for AD researchers to focus their efforts on finding new ways to prevent both the accumulation of amyloid-β and the degradation of the blood-brain barrier.

As NSC therapy shows great promise for treating neurological disorders, it is thought that the facilitation of neural recovery by this method offers the greatest chance of defeating AD. In addition to replacing dead or injured brain cells, NSCs' previously discussed use of the bystander effect in addition to microenvironment modulation showcase this method's ability to combat many of the precipitating factors of AD (Lee et al., 2007). More specifically, treatments for AD are focusing on two different avenues: delivering therapeutics during the chronic stage of AD to alleviate already developed symptoms and delivering NSCs during the early stages of neurodegeneration with the goal of NSC migration to injury sites to combat early AD pathology (Boese et al., 2020). The avenue that aims to treat the chronic symptoms of AD would be critical for those currently suffering from AD-induced neurodegeneration. To repair neurodegeneration that has already occurred, NSCs' ability to rapidly proliferate and replace damaged cells would be critical. Additionally, NSCs' secretion of therapeutic products could potentially lead to

increased functioning of the blood-brain barrier, which would contribute to limiting the damage caused by amyloid-β buildup. The second avenue, treating the early onset of AD, requires researchers to be able to identify the signs of developing neurodegenerative disease. A few of these critical biomarkers, which have been linked to AD development, are the concentration of amyloid-β in cerebrospinal fluid (CSF) and blood and the imaging of tau deposits by positron emission tomography (PET; Brier et al., 2016). With these methods, clinicians of the future may be able to screen those with genetic dispositions for AD and schedule at-risk individuals for NSC therapy.

As with most novel scientific developments, there are currently limitations to using NSCs to treat AD. One of the most cumbersome of these limitations is the previously mentioned inability to fully control the cell types of NSC differentiation. While it has been found that NSC therapy leads to the generation of neurological tissue, in many cases, animal trials have seen NSC implantation stimulate unwanted differentiation into non-neural cell types and uncontrolled generation of glial cells (Xuan et al., 2009). It is essential that these complications are resolved before humans can hope to be treated by NCS therapy, as the overproduction of glial cells could lead to severe complications, such as inflammation. Another complication that has been discovered during NSC research is the decline of NSC content in the brain with age (Manganas et al., 2007). If not resolved, this could cause a person undergoing NSC treatment to need many grafts of stem cells before seeing results and could even limit the efficacy of using NSCs to treat some individuals.

Parkinson's Disease

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by bradykinesia, rigidity, and tremor. Generally, these symptoms are caused by the gradual loss of

nigrostriatal dopamine (DA) neurons and the subsequent neuronal degeneration. While the current treatments that exist for PD have been successful at relieving a range of symptoms, no widespread treatment exists to counteract the progression of this disease. With the ever-increasing popularity of research focusing on using stem cells and tissue transplants to counter disease, current studies are working to explore these avenues for PD treatment.

One strategy that has yielded results has been to provide patients with intrastriatal grafts of human fetal ventral mesencephalic (hfVM) tissue. This has shown that grafted tissue of DA-producing neurons can replace dead DA neurons by transplantation (Politis & Lindvall, 2012). With this method, grafts can provide a population of DA-producing neuronal cells that lasts up to 16 years and have been known to provide some symptomatic relief. While this sounds promising, the results from studies involving hfVM grafts have been inconsistent and hindered by the creation of adverse effects such as graft-induced dyskinesias (GIDs). Additionally, there are considerable ethical dilemmas amongst researchers on the use of human fetal stem cells for disease treatment. Because of these drawbacks, different methods of treating PD are still needed to gain widespread support. Stem cells could potentially solve this problem by providing an ethical and endless supply of DA neurons for transplantation.

To develop methods of treating PA with stem cells, researchers have used gene modification technology to reprogram adult fibroblasts into induced pluripotent stem cells. Once these iPSCs are formed, they can differentiate into DA-ergic neurons, raising the possibility of generating an unlimited supply of replacement neurons for PA patients (Politis & Lindvall, 2012). To test this method of replacing DA-ergic neurons, researchers first transplanted iPSCs into the striatum of a PD rat model. After evaluation with MRI technology, the animal test subjects showed some degrees of functional recovery after receiving the iPSC transplants. In

addition to the apparent success of this method in an animal PD model, an additional advantage of using iPSCs is that they can be catered to specific patients. However, a downside of iPSCs is that there have been concerns over the formation of tumors in some test animals after receiving iPSC transplants. Because of this, more tests need to be done so reliable and safe methods of treating PD with iPSCs are developed and transitioning to a clinical setting is viable.

Another possible source of stem cells to treat PD is the bone marrow. Bone marrow-derived mesenchymal stem cells have been reported to differentiate into neurons that are tyrosine hydroxylase-positive and improve motor performance in mice (Politis & Lindvall, 2012). Recently, this research has been expanded into a clinical setting, where a new trial involving advanced PD patients attempted to discover the abilities of transplanted MSCs to differentiate into DA-ergic neurons. In the study, PD patients received unilateral transplantations of analogous bone marrow-derived MSCs into the sublateral ventricular zone of the brain. After 12 months, the patients reported modest clinical improvement and showed no signs of tumor formation. Because the research in this trial did not involve collecting MRI or PET scan data before and after MSC treatment, the mechanisms responsible for these improvements are unfortunately unknown. Hopefully, future clinical studies designed to treat PD with neurons derived from MSCs will be able to gain more data on these cells' abilities.

Although hfVM tissue transplants, iPSCs, and MSCs have all demonstrated the ability to restore limited amounts of functionality in PD models, PD stem cell research is currently focused on creating standardized DA-ergic neuroblasts from stem cells. To be successful, it needs to be demonstrated that the neuroblasts developed for PD treatment cannot only replace lost DA-ergic neurons but also mitigate the chances of adverse side effects, such as tumor development and

GIDs (Politis & Lindvall, 2012). If these hallmarks are met, the first demonstration of using stem cells as an effective restorative treatment for PD patients could soon be seen.

Neurotrophins and Alzheimer's Disease

Neurotrophic factors serve important roles in the central and peripheral nervous systems as mediators of cell growth, survival, and homeostatic functions. Of these factors, a few of the most influential are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IFG-1), and glial cell line-derived neurotrophic factor (GDNF) (Marsh & Blurton-Jones, 2017). Unsurprisingly, it has been shown that these important neurotrophic factors are decreased in AD patients and animal models. Because of this, research has begun to investigate the effects of restored levels of neurotrophic factors in AD models to see increased neuronal health, learning, and memory.

One challenge with this idea is that when delivered by direct injection, many neurotrophic factors cannot cross the blood-brain barrier. To resolve this issue, it has been suggested that NSCs could serve as an effective delivery method since they produce a variety of neurotrophic factors themselves and also migrate extensively (Marsh & Blurton-Jones, 2017). To test this, researchers used a murine model of AD that exhibited both β-amyloid and tau pathology. After transplanting NSCs directly into the hippocampus, researchers found that mouse learning and memory had improved after the observation period. While some may suggest these results are attributed to the NSCs themselves, researchers in this study found that the test mice showed significantly elevated brain levels of BDNF after NSC transplantation. Additionally, in a second trial where AD mice received NSCs with limited BDNR producing abilities (via shRNA-mediated knockdown), it was discovered that the previously recorded cognitive and functional restorations were no longer present. In lieu of these results, a subsequent

study was developed where a different AD model tested the same variables (Zhang et al., 2014). Again, it was found that transplanted NSCs had no intrinsic effects on Aβ pathology; however, cognitive restoration was observed in step with increased BDNF levels. From the combination of these studies, it is clear that in multiple animal AD models, cognitive recovery was observed following NSC facilitation of BDNF increase.

Since the above results were largely found in AD models where Aβ amyloid accumulation was the observed pathology, additional research has been conducted on a mouse model displaying accumulation of hyperphosphorylated tau, a common AD pathology, (Hampton et al., 2010). To investigate whether increased neurotrophic factors could also lead to cognitive recovery in this model, researchers in this study transplanted NSCs into the cortex of the tau AD model. It was found that after NSC transplantation, neuronal loss had been reduced in comparison to the vehicle treatment. However, this result was not likely due to direct neuronal differentiation and replacement. This is because the majority of transplanted NSCs were found to have differentiated into astrocytes, therefore not explaining the observed neuronal recovery. To confirm that direct neuronal replacement was not the source of reduced neuronal loss, the trials were repeated using astrocytes (no differentiation capabilities) instead of NSCs, and it was found that neuronal loss was reduced to a similar degree. After these trials were completed, researchers were able to examine mRNA levels of key neurotrophic factors and found that while BDNF and NGF were unchanged, GDNF levels had been increased 4-fold after NSC transplantation and when compared to the vehicle. This suggests that the neurotrophic factor GDNF may also be essential to limiting the loss of neurons in tau AD models. As demonstrated by the previously described studies, it is clear that neurotrophins play essential roles in neurological recovery and that NSCs show promise as an effective delivery method in AD models. Further investigation

into this area should focus on developing NSCs that produce high levels of neurotrophins and therefore may yield greater degrees of neurological recovery.

Neurotrophins and Parkinson's Disease

In a similar way to AD, PD is also known to exhibit the loss of neurotrophic factors in the brain. Of these neurotrophic factors, BDFN and GDNF, specifically, have been shown to affect the survival and function of DA neurons in the midbrain. If the levels of these key neurotrophic factors can be restored to normal levels, it is hopeful that DA neuronal loss can be limited and restored in PD patients.

Several studies designed to test this theory used NSCs with greater than normal GDNF expression ($GDNF^{++}$) to enhance DA neuron transplantation. In one study, a rat model of PD was given transplants of DA neurons and $GDNF^{++}$ NSCs and it was found that post-transplantation, the rats exhibited decreased DA neuronal degeneration (Akerud et al., 2001). Because of these results, subsequent studies have been conducted to investigate whether human NSCs modified to express different neurotrophins can both protect DA neurons and limit motor deficits. It was found that transplanting NSCs with an overexpression of IGF-1 and GDNF resulted in reduced DA neuronal loss (Ebert et al., 2008). Additionally, while it was discovered that significantly more NSCs that were modified to express IGF-1 survived the transplantation process than those that were modified to express GDNF, both grafts of NSCs resulted in similar neuronal survival and behavioral recovery (Marsh & Blurton-Jones, 2017). This suggests that GDNF may be a more potent influencer of neuronal survival.

While the animal trials described in these studies show that neurotrophin-producing NSCs are indeed effective at preventing DA neuronal degradation, it is important to note that the majority of clinical PD cases show an α-synuclein pathology which is not shown in these trials

and is likely to influence PD pathology. To investigate this complication, a novel study involving an animal model of synucleinopathy showed that after receiving BDNF-producing NSC transplants, both motor and cognitive recovery was observed (Goldberg et al., 2015). While the animal model used for this trial was more representative of the neurological disorder, dementia, the results undoubtedly show that neurotrophic factors have a large effect on motor and cognitive recovery in an animal model of synuclein pathology. In combination with the PD model NSC/neurotrophin studies, the results of this research show that neurotrophins are of great importance for the future of PD research.

Application for Stroke Recovery

Stroke, which occurs when blood supply to part of the brain is interrupted, leading to dead and damaged neural tissue, is the primary cause of long-term disability in the United States and the second leading cause of death worldwide. In the wake of this massive epidemic, a large wave of clinical trials has begun to test the effectiveness of different therapeutics. Unfortunately, only tissue plasminogen activator (tPA) is FDA approved to treat stroke in humans (Baker et al., 2017). The largest problem with this specific treatment is that it is only viable for treating one type of stroke (ischemic stroke) and also serves primarily to limit ischemic damage. tPA currently has no regenerative functions that would allow patients to achieve neurological recovery from restored brain tissue. Since the scope of tPA is heavily limited, new methods of treatment for stroke patients must be found to resolve this issue.

A recent study involving induced pluripotent stem cells (iPSCs) has shown that these stem cells can be generated from a patient's somatic cells. From there, they model similar plasticity to embryonic stem cells and therefore can differentiate into induced neural stem cells (iNSCs) (Baker et al., 2017). Previous research has shown these neural stem cells are capable of

effectively bringing about neurological recovery in rodent models of ischemic stroke. They do this in two different ways: by differentiating into neurons that replace those lost due to stroke neurological damage, and by producing regenerative and neuroprotective factors that affect parenchymal healing. In this rodent model, researchers found transplanted iNSCs capable of reducing tissue atrophy, promoting angiogenesis, inducing glial scar formation, and promoting many different mature neuronal cell types. While these results seem very promising, key functional and physiological differences exist between the rodent brain and the human brain, making the results of this study questionable for human application. In particular, human brains contain prominent differences from rodent brains in their gray to white matter composition, blood flow, gyral patterning, and metabolism. These areas are widely affected by stroke, and therefore, it is important to achieve results in model animals that will show a direct correlation to humans.

In a different study designed to test a new animal model for stroke, pigs were selected for a stroke therapy model. Compared to the rodent brain, the pig brain contains much more similar anatomical and physiological structures to that of the human brain, including those important characteristics previously described (Baker et al., 2017). In the trials of this study, iNSCs were first transplanted into the stroke model pigs before non-invasive longitudinal magnetic resonance imaging (MRI) was performed. The results of this screening showed that after iNSC injection, stroked animals showed improvements in white matter integrity, brain metabolism, and cerebral blood perfusion. In addition to these discoveries, it was also found that iNSC therapy yielded neurogenesis, neural protection, and decreased microglial activation. For the first time, a transitional large animal model demonstrated that iNSCs show great regenerative and restorative potential.

Clinical Trials

In response to the wealth of data gained from animal trials of neural stem cell transplantation, certain clinical trials have been approved for using NSCs to treat ischemic stroke in humans. One of these trials, the CTX Pilot Investigation Stem Cells in Stroke (PISCES) Phase I has recently been completed in the first human trial of stem cell therapy to treat stroke in the United Kingdom (Sinden et al., 2017). Because of the risks associated with transplanting any outside organic material into the human body, this trial was only viable after extensive animal trials and safety tests. These animal trials largely utilized rats with chronic stroke and demonstrated improvements in behavior and long-term safety. In the PISCES trial, men of at least 60 years of age who were living with chronic stroke were able to receive doses of CTX NSCs in amounts of 2, 5, 10, or 20 million by stereotactic ipsilateral putamen injection. In this pilot human trial, researchers were primarily interested in assessing the safety and tolerability of CTX transplants as well as observing any neurological and functional outcomes in patients over 24 months.

After collecting data over the trial period of 24 months, researchers of the PISCES Phase I trial reported no cell-related adverse events in patients. From imaging during the CTX injection procedure, serious adverse events (SAEs) were reported in 4 out of 11 patients. Of these SAEs, none were discovered to be symptomatic (Sinden et al., 2017). While previous trials of neuronal cell implantation have raised concerns about causing seizures in patients, no such events were found in any patients during the Phase I trial of CTX, an initially promising result.

After CTX NSC transplantation was complete, numerous scales were used to monitor efficacy in patients. Among these scales were a modified Rankin Scale (mRS), NIH stroke scale (NIHSS), Ashworth Scale for upper- and lower limb spasticity, and the Barthel Index of

activities of daily living (BI) (Sinden et al., 2017). Using these scales, patients were assessed closely for any improvements in the above criteria. Following CTX implantation, it was found that statistically significant improvements were seen in NIHSS, and non-significant improvements were seen in the summed scores of the arm and leg Ashworth scale and the BI index. While the improvements displayed by these scaled are remarkable, not every patient in this trial showed signs of recovery. In 7 out of 11 patients, disability, as measured by the mRS was found to be unchanged after 12 months, and in the remaining 4 patients, it only improved by one grade. At the end of the trial period of 24 months, disability was still unchanged in 7 patients, was worsened by two grades in one patient, and had improved by one grade in three patients.

In addition to the scale used by researchers to monitor results, patients in this study also had the option to report their overall health statuses. When compared to the baseline, patients reported that their health had improved by a median of 18 points (interquartile range -5 to 30) after 12 months (Sinden et al., 2017). As an additional method of gathering data, functional magnetic resonance imaging (fMRI) was also used both pre and post-treatment to screen for neurological developments. From this data, it was found that changes in patients' motor activation were consistent with fMRI measured neurological improvement.

Since the PISCES Phase I trial involved a very small number of treated individuals, a heterogeneous study population, and a single-arm design, reliable conclusions could not be drawn about neurological and functional recovery after CTX NSC implantation. Any such conclusion would apply only to the population of individuals tested and would need more trials involving outside controls to avoid confounding factors. This being said, it is worthwhile to note that despite the trial's population of patients being in the late stages of chronic stroke, many of

the individuals treated in this phase showed improvement in different areas (Sinden et al., 2017). While it is unknown whether these improvements can be linked to stem cell implantation or outside factors such as increased medical attention, this study seems to cast doubt on the regular assumption among clinicians that late-stage chronic stroke cannot be treated. In addition to the improvements documented above, it should also be noted that multiple patients in Phase I of this trial were found to report occasional and unmeasured instances of minor return of finger movement, improved visual perception, reduced spasticity, and better bed-to-chair transfers. It is hopeful that in later phases of the PISCES trial, measures will be taken to ensure that these improvements can be quantified and reported.

In this pilot trial, men, as opposed to a mixed group of men and women, were selected and enrolled, due to the lack of reproductive toxicology reports for stem cell studies of any origin. This was especially relevant to this trial where a tamoxifen metabolite analog receptor is used to control cell manufacture of CTX (Sinden et al., 2017). While tamoxifen has been found to affect the female reproductive system in other cases, future clinical studies of CTX may involve female participation as preclinical studies of CTX NSC transplantation in both male and female stroke animal models did not show any negative events. In addition to the lack of mixed-gender participants, a unique feature of this trial was a lack of immunosuppressive therapy. This was supported by the knowledge that nonclinical studies of CTX showed no evidence that immunosuppression would be required for either cell survival or differentiation. Further, immunosuppression was seen as an unnecessary risk as it has been previously linked to infection after stroke (Stroemer et al., 2009). This, with the knowledge that test subjects in this trial were individuals with preexisting conditions, made limiting the risk of infection an essential component of Phase I.

Due to successes in the demonstration of safety and in obtaining useful data in the Phase I trial of PISCES, a second trial, Phase II, was formed. In this second clinical trial, patients who were experiencing single-arm paresis after suffering an ischemic stroke were able to receive a single dose of 20 million CTX NSCs (Sinden et al., 2017). This phase tested the feasibility of using CTX to treat single-arm paresis over 12 months and showed that a sufficient proportion of the test population experienced some functional recovery. The study also determined that CTX treatment was both safe and tolerable, with the majority of adverse side effects reported by patients being related to the surgical procedure itself (Muir et al., 2020). To test the results of this study, similar scales of recovery as used in PISCES Phase I were incorporated, including the NIHSS, a more objective version of mRS, and BI. PISCES Phase II, being a successful study of CTX treatment for single-arm paresis after ischemic stroke, will pave the way for a subsequent larger clinical study, PISCES III, which is currently in development.

Conclusion

By replacing damaged neural tissue, fighting inflammation and immune responses, delivering neurotrophic factors to areas of deficit, and much more, neural stem cells show remarkable abilities that are not seen in other cells of the body. Although neurological disease is currently widespread in both the United States and the rest of the world, it is hopeful that one day, NSC research may allow those suffering from disorders such as Alzheimer's disease, Parkinson's disease, and stroke to finally experience recovery. While more research needs to be conducted before this goal becomes a reality, the rate of discovery in neural stem cell research over the last couple of decades is astonishing. With this in mind, it is within reason to hope that breakthroughs in NSC research will continue to be made in the next decade, and the one after that, until neurological disease is a concern of the past.

References

Akerud et al., (2001). Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. *Journal of Neuroscience, 21*, pp. 8108-8118.

Alison et al., (2002). An introduction to stem cells. *The Journal of Pathology, 197*(4), 419-423. https://10.1002/path.1187.

Baker et al., (2017). Induced pluripotent stem cell-derived neural stem cell therapy enhances recovery in an ischemic stroke pig model. *Scientific Reports,7,*10075. https://10.1038/s41598-017-10406-x.

Boese et al., (2018). Neural stem cell therapy for subacute and chronic ischemic stroke. *Stem Cell Research and Therapy, 9:154.* https://10.1186/s13287-018-0913-2.

Boese et al., (2020). Neural stem cell therapy for neurovascular injury in Alzheimer's disease. *Experimental Neurology, 324*, 113112. https://10.1016/j.expneurol.2019.113112.

Brier et al., (2016). Tau and Abeta imaging, CSF measures, and cognition in Alzheimer's disease. *Science Translational Medicine, 8*, 338 – 366.

Cardoso et al., (2018). Target-specific forebrain projections and appropriate synaptic inputs of hESC-derived dopamine neurons grafted to the midbrain of parkinsonian rats. *Journal of Comparative Neurology, 526:*2133–2146. https://10.1002/cne.24500.

Denker, (2021). Autonomy in the Development of Stem Cell-Derived Embryoids: Sprouting Blastocyst-Like Cysts, and Ethical Implementations. Cells, *10*(6), 1461; https://doi.org/10.3390/cells10061461.

Ebert et al., (2008). Human neural progenitor cells over-expressing IGF-1 protect dopamine neurons and restore function in a rat model of Parkinson's disease. *Experimental*

Neurology, 209, pp. 213-223.

Frotscher, (1992). Specificity of interneuronal connections. *Annals of Anatomy, 174*(5), 377-382. https://10.1016/S0940-9602(11)80254-2.

Goldberg et al., (2015). Neural Stam Cells Rescue Cognitive and Motor Dysfunction in a Transgenic Model of Dementia with Lewy Bodies through a BDNF-Dependent Mechanism. *Stem Cell Reports, 5*: 791-804. doi: 10.1016/j.stemcr.2015.09.008.

Haas et al., (2005). Adult stem cell therapy in stroke. *Current Opinion in Neurology,18:*59–64.

Hampel et al., (2021). The Amyloid-β Pathway in Alzheimer's Disease. *Molecular Psychiatry, 26*, 5481–5503.

Hampton et al., (2010). Cell-mediated neuroprotection in a mouse model of human tauopathy. *Journal of Neuroscience, 30*, pp. 9973-9983.

Hardy & Selkoe. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*, 353-356.

Hebert et al. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology, 80*, 1778-1783.

Ladewig et al., (2014). Auto-attraction of neural precursors and their neuronal progeny impairs neuronal migration. *Natural Neuroscience, 17*, 24–26. doi: 10.1038/nn.3583.

Lee et al., (2007). Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nature Medicine, 13*, 439-447.

Lee et al., (2008). Anti-inflammatory mechanism of intravascular neural stem cell transplantation in hemorrhagic stroke. *Brain, 131*, (616-629). doi:10.1093/brain/awm306.

Li et al., (2007). Critical issues of clinical human embryonic stem cell therapy for brain repair. *Trends in Neurosciences, 31*(3), 146-153. https://10.1016/j.tins.2007.12.001.

Li et al., (2014). In vivo assessment of guided neural stem cell differentiation in growth factor immobilized chitosan-based hydrogel scaffolds. *Biomaterials, 35* (33), 9049-9057. https://10.1016/j.biomaterials.2014.07.038.

Lindvall et al., (1992). Transplantation of fetal dopamine neurons in Parkinson's disease: One-year clinical and neurophysiological observations in two patients with putaminal implants. *Annals of Neurology, 31:*155-165.

Manganas et al., (2007). Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. *Science, 318*: 980–985.

Marsh & Blurton-Jones, (2017). Neural Stem Cell Therapy for Neurodegenerative Disorders: The Role of Neurotrophic Support. *Neurochemistry International, 106*: 94-100. doi: 10.1016/j.neuint.2017.02.006.

Muir et al., (2020). Intracerebral implantation of human neural stem cells and motor recovery after stroke: multicentre prospective single-arm study (PISCES-2). *Neurology, Neurosurgery, and Psychiatry, 91*(4). http://dx.doi.org/10.1136/jnnp-2019-322515.

Payne et al., (2018). In Vitro Maturation of Human iPSC-Derived Neuroepithelial Cells Influences Transplant Survival in the Stroke-Injured Rat Brain. *Tissue Engineering, 24*: 3-4. doi: 10.1089/ten.tea.2016.0515.

Politis & Lindvall, (2012). Clinical application of stem cell therapy in Parkinson’s disease. *BMC Medicine, 10* (1), 1. https://10.1186/1741-7015-10-1.

Punt et al., (2019). *Kuby Immunology*. W.H. Freeman/Macmillan Learning.

Salado-Manzano et al. (2020). Is the immunological response a bottleneck for cell therapy in neurodegenerative diseases? *Frontiers*. Retrieved December 7, 2021, https://www.frontiersin.org/articles/10.3389/fncel.2020.00250/full.

Sinden et al., (2017). Human neural stem cell therapy for chronic ischemic stroke: Charting progress from laboratory to patients. *Stem Cells and Development, 26* (13), 933-947. https://10.1089/scd.2017.0009.

Snow et al., (2019). A phase IIb, randomized, double-blind, placebo-controlled, dose-ranging investigation of the safety and efficacy of NTCELL® [immunoprotected (alginate-encapsulated) porcine choroid plexus cells for xenotransplantation] in patients with Parkinson's disease. *Parkinsonism and Related Disorders, 61*, (88-93). https://doi.org/10.1016/j.parkreldis.2018.11.015.

Stroemer et al., (2009). The neural stem cell line CTX0E03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. *Neurorehabilitation and Neural Repair 23*: 895–909.

Swijnenburg et al., (2008). Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proceedings of the National Academy of the Sciences, 105*, (12991-12996). www.pnas.org/cgi/doi/10.1073/pnas.0805802105.

Wei et al., (2021). Immunological Responses to Transgene-Modified Neural Stem Cells After Transplantation. *Frontiers in Immunology, 12*, (1-12). https://doi.org/10.3389/fimmu.2021.697203.

Xuan et al., (2009) Effects of engrafted neural stem cells in Alzheimer’s disease rats. *Neuroscience Letters, 450*: 167–171.

Zhang et al., (2014). Neural stem cell transplants improve cognitive function without altering

amyloid pathology in an APP/PS1 double transgenic model of Alzheimer's disease. *Molecular Neurobiology, 50,* pp. 423-437.