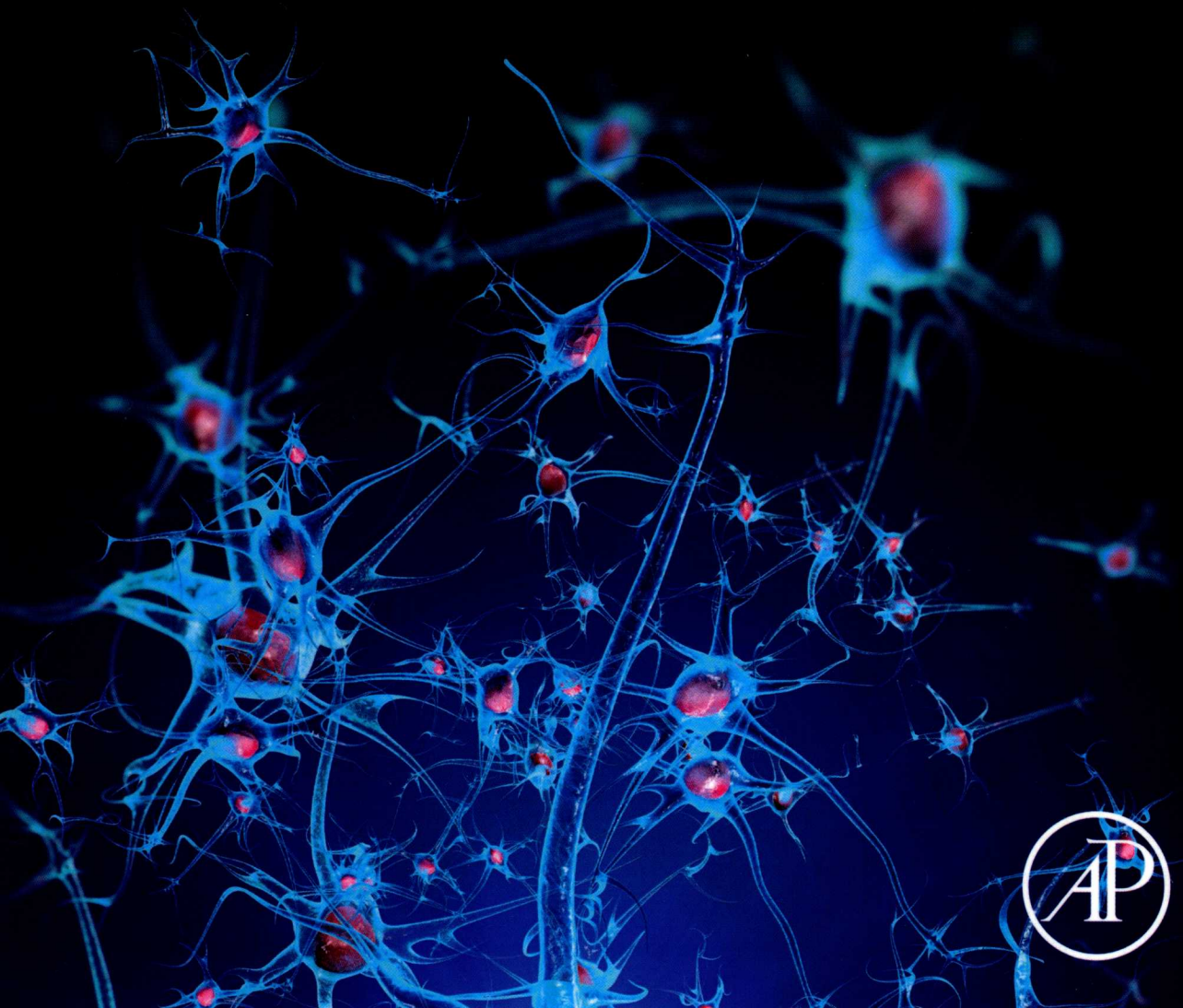
Engineering Neural Tissue from Stem Cells

Stephanie Willerth



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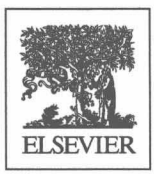

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Author Biography

Stephanie Willerth currently holds a Canada Research Chair in Biomedical Engineering at the University of Victoria where she is dually appointed in the Department of Mechanical Engineering and Division of Medical Sciences as an Associate Professor. Her interdisciplinary research group investigates how to engineer neural tissue by combining pluripotent stem cells, controlled drug delivery, and biomaterial scaffolds. Her honors include being named a Women of Innovation in 2017, a 2015 Young Innovator in Cellular and Molecular Biology, a Star in Global Health by Grand Challenges Canada, and the 2014 Faculty of Engineering Award for Excellence in Teaching. She is an active member of the Stem Cell Network and the International Collaboration on Repair Discoveries, who supported her 2016 sabbatical leave at the Wisconsin Institute for Discovery where she authored this book. She served as both the Director of the Centre for Biomedical Research and the President of the Canadian Biomaterials Society during 2017. Before accepting her faculty position, Willerth completed a National Institutes of Health sponsored postdoctoral fellowship at the University of California—Berkeley and graduate studies at Washington University. She received undergraduate degrees in Biology and Chemical Engineering from the Massachusetts Institute of Technology.

Foreword

Dr. Willerth has brought together the main themes underlying tissue engineering in the central nervous system, with a focus on the brain, the spinal cord and including both traumatic and degenerative diseases. The book follows a natural progression: the first three chapters set the stage with a description of the central nervous system, its diseases and disorders, and the stem cells used therein; the subsequent three chapters, 4–6, focus on the role of biomaterials and tissue engineering in overcoming these diseases in conjunction with stem cells; the last two chapters, 7 and 8, highlight the benefit of therapeutics co-delivered with stem cells and an outlook to the future.

In what many consider the final frontier of medicine, the central nervous system has been a formidable challenge, with a series of biological barriers making access and regeneration more difficult. It is important to realize that there is often very little that can be done for patients suffering from traumatic injuries such as stroke and spinal cord injury. For stroke, tissue plasminogen activator is the only approved treatment and it must be administered within 4 h to the patient. In spinal cord injury, methylprednisolone is administered to patients, but its benefit has been questioned. Importantly, a recent clinical trial led by Asterias has shown very promising results in spinal cord injury with the transplantation of oligodendrocyte progenitor cells derived from embryonic stem cells. There is also excitement about ongoing clinical trials for degenerative diseases like blindness due to age-related macular degeneration.

Whether transplantation of exogenous stem cells or stimulation of endogenous stem cells, biomaterials can have a profound impact on success. In both cases, cell survival and integration are key barriers. Biomaterials can provide the environment that promotes greater cell survival by manipulating the mechanical and biochemical properties. Similarly, biomaterials can be used to deliver biomolecules locally to the brain or spinal cord, to stimulate endogenous stem cells and/or promote integration by breaking down barriers, such as that formed by the glial scar. With this book, Dr. Willerth captures some of the key challenges and opportunities that face engineering neural tissue in the central nervous system with stem cells.

Molly S. Shoichet
University of Toronto

Acknowledgments

It takes a lot of time and support to write a book, here is where I attempt to compile the list of people who supported me throughout this project and show my appreciation.

First, I must thank everyone at Elsevier for their support, including my editors, Anna Valutkevich and Chris Katsaropoulos, along with my permissions team, Narmatha Mohan, Vinoth Kumar Balu, and the production staff.

I am thankful for the members of Willerth lab past and present for inspiring this work. I would like to highlight Meghan Robinson, Krista Wilson, Michaela Thomas, Laura de la Vega, and Andrew Agbay for their constructive feedback on the contents of this book.

My research group has received funding from various agencies, enabling my group to conduct neural tissue engineering research. This research, in turn, served as an inspiration for this project. These funding sources include the Canada Research Chairs program, the Natural Sciences and Engineering Resource Council, the Stem Cell Network, the Rick Hansen Institute, and Grand Challenges Canada. Additional support for this project came from the International Collaboration on Repair Discoveries and the Rick Hansen Institute, which sponsored my sabbatical leave at the University of Wisconsin through their International Exchange Award program. I would like to thank the Wisconsin Institute for Discovery at the University of Wisconsin—Madison for hosting me with special thanks to Randolph Ashton and Krishanu Saha. It provided an inspiring environment where I wrote majority of these books. I would like to thank my colleagues at the University of Victoria in the Department of Mechanical Engineering and in the Division of Medical Sciences, especially Kurt McBurney for his feedback. This book was also inspired by my students at the University of Victoria with special acknowledgements to the UVic BioDev student club and the UVic Formula Motorsport team that I advise.

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In terms of inspiration outside of science, my nephews, Tommy Willerth, Leo, and Boston Lukosky, are always amusing along with my beloved Kansas City Royals and the United States Women's National Soccer team. Special thanks to Formula One and NASCAR along with my favorite drivers Jenson Button and Brad Keselowski for keeping me amused during the lengthy writing process. I would also like to thank my housemates during my time in Madison—Glenn Trudel and his cat Lilly—for providing a creative environment.

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Last but not the least, I would like to thank my husband, Gary Escudero, for his constant support in putting up with me and my academic tendencies since 1999.

Contents

CHAPTER

The need for engineering neural tissue using stem cells 1

1 OVERVIEW OF THE NERVOUS SYSTEM

Our nervous system controls all of our actions, including those that are voluntary and those that are involuntary [1]. The complex nature of the nervous system enables us to sense and respond to our environment. Fig. 1 shows the different components that compose the nervous system, including the central nervous system and the peripheral nervous system. The central nervous system consists of the brain and spinal cord, while the peripheral nervous system contains the nerves located outside of these two organs. Several important differences exist between the central and the peripheral nervous system, which will be explored in depth in Chapter 2. In general, the central nervous system possesses a lower capacity for regeneration, while the peripheral nervous system has a higher capacity for regeneration after injury. Proper function of the nervous system requires that these tissues and organs retain their important structural features as well as the metabolic functions of the various cell types. Disruptions of these structures and their associated functions can have devastating consequences, leading to manifestation of diseases and disorders of the nervous system. This chapter will introduce some of the most common diseases and disorders that affect the nervous system along with the current treatment options and how stem cells could potentially cure these disorders.

2 DEMAND FOR CURES FOR NEURODEGENERATIVE DISEASES AND DISORDERS USING NEURAL TISSUE ENGINEERING

As the population ages, the costs associated with the healthcare required to treat patients with diseases and disorders will also continue to rise along with demand for treatments. This section will give a brief overview of the most common nervous system diseases and disorders along with the current state of treatments associated with them. Table 1 summarizes some of the major diseases and disorders that afflict the central nervous system and their associated healthcare burden. I have chosen to focus on the statistics of those suffering from these diseases and disorders in the United States and Canada due to their availability. A list of the websites for the foundations and other relevant organizations discussed in this chapter is given at the end of this chapter. Most of the treatments for these neurological diseases and disorders only alleviate the symptoms and do not represent a cure as detailed in

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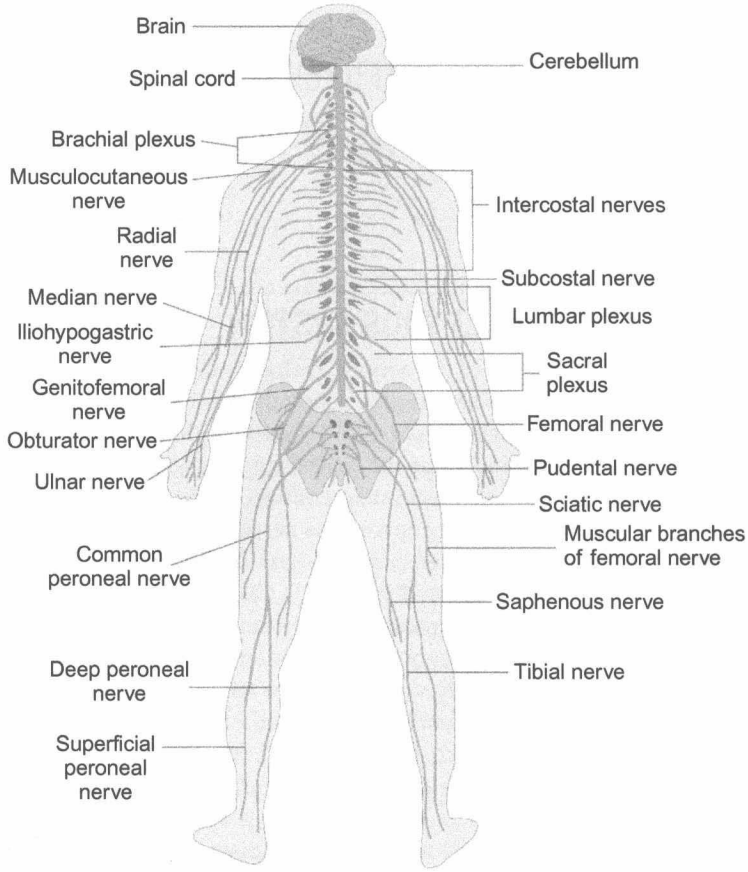

FIG. 1

Schematic of the nervous system. The brain and spinal cord comprise the central nervous system while the other nerves comprise the peripheral nervous system.

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the following sections. This book will explore how stem cells could potentially be used to develop cures for such complex diseases and disorders. Alzheimer's disease remains the most common neurological disease in North America with other major diseases of the central nervous system being Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis. A significant healthcare burden also exists due to injuries to the central nervous system, such as traumatic brain injury and spinal cord injury. Fig. 2 illustrates the relative incidence of each of these diseases and disorders in the form of a word cloud for easy visualization. The font size of each disease and disorder listed is proportional to the number of people suffering from that particular issue.

Table 1 List of the Most Common Neurological Diseases and Disorders Along With Relevant Statistics

Disease/Disorder	Number of People Suffering	Total Healthcare Burden	Source
Alzheimer's disease	~5,000,000 (USA)	~$236,000,000,000	Alzheimer's Association
	~564,000 (Canada)	~$10,400,000,000	Alzheimer Society Canada
Parkinson's disease	~1,500,0000 (USA)	~$25,000,000,000	Parkinson's Disease Foundation
	~100,000 (Canada)	~$558,100,000	Parkinson Canada
Huntington's disease	~30,000 (USA)	~$600,000,000[a]	Huntington's Disease Society of America
	~5000 (Canada)	~$100,000,000[a]	[2]
Amyotrophic lateral sclerosis	~20,000 (USA)	~$256–43,300,000	ALS Association/Muscular Dystrophy Association
	~3000 (Canada)	~$150,000,000	ALS Canada
Multiple sclerosis	~350,000–500,000 (USA)[b]	Not available	National MS Society
	~100,000 (Canada)	Not available	MS Society Canada
Traumatic brain injury	~1,700,000 annually (USA)	~$60,000,000,000	Center for Disease Control
	~1,000,000 (Canada)	Not available	Brain Injury Canada
Spinal cord injury	~282,000 (USA)	~$60,000,000,000	National Spinal Cord Injury Statistical Centre
	~86,000 (Canada)	~$2,700,000,000	Rick Hansen Foundation

[a]*Based on an average cost per patient of $20,000.*
[b]*The National MS Society is currently performing a study that will more accurately determine the prevalence of this disease.*

FIG. 2

Word Cloud showing the relative incidences of the diseases and disorders of the central nervous system based on font size. Alzheimer's disease is the most common nervous system disorder in terms of patient numbers.

The diseases and disorders of the nervous system have generated significant interest in finding cures from both government and private organizations. For example, President Obama launched the White House BRAIN Initiative (Brain Research through Advancing Innovative Neurotechnologies) in 2013. The overall goal of this 12-year initiative is to develop the tools and technologies for understanding how the brain works. This effort has led to comparisons with the Human Genome Project, which greatly advanced our understanding of genetics and biology by sequencing the human genome. In Canada, private organizations, such as the Brain Canada Foundation and the W. Garfield Weston Foundation, support research in understanding how the brain works and how these different neurological diseases could be treated. These diseases and disorders of the nervous system are often complicated in terms of their biology, and they will require significant interventions to cure as detailed in the following sections.

2.1 ALZHEIMER'S DISEASE

Alzheimer's disease represents one of the most common neurological diseases in North America. Approximately one out of every nine Americans over the age of 65 suffers from this disease with the associated healthcare costs estimated to be $246 billion each year for the United States and Canada as reported by the Alzheimer's Association and the Alzheimer Society Canada. Alzheimer's disrupts the normal connections between brain cells, resulting in dementia. It is characterized by the presence of plaques and tangles in the tissue of the brain along with the degradation of cells known as basal forebrain cholinergic neurons [3]. While a positive diagnosis can currently only be confirmed postmortem based on tissue analysis, patients can be given cognitive tests to assess their mental state in terms of dementia, which occurs when normal brain function is disrupted [4]. More recently, scientists have been investigating different modalities for imaging brain tissue to detect the presence of the plaques and tangles associated with Alzheimer's. While the disease is not directly fatal, the effects of dementia lead to other physical issues, such as an inability to balance and move, which in turn, lead to fatal complications. The current

Food and Drug Administration approved treatments for Alzheimer's disease include five pharmacological drugs that regulate brain chemistry to restore proper communication in the brain that was lost due to dementia. These treatments include four types of cholinesterase inhibitors and one type of NMDA (*N*-methyl-D-aspartate) receptor antagonist [5]. Both drugs modulate the levels of neurotransmitters, chemicals released by nerve cells that enable communication with other nerve cells. These medications have varying degrees of effectiveness depending on the patient, and they only treat the symptoms of this disease. They do not treat the underlying neuronal degeneration that occurs, making these treatments short-term ways of dealing with this disease. Stem cells could potentially offer a long-term cure for this disease although much work remains before such therapies could be implemented [6,7]. For example, a certain subset of stem cells known as mesenchymal stem cells modulate the immune system of the body by eliminating foreign agents. These stem cells can potentially clear the plaques and tangles associated with Alzheimer's disease as a way to eliminate their effects on the nervous system and restore proper function [8].

2.2 PARKINSON'S DISEASE

Parkinson's disease causes degeneration of the central nervous system, resulting in impaired motor skills followed by cognitive defects as the disease progresses. The Canadian actor Michael Fox suffers from this disease and has established the Michael J. Fox Foundation for Parkinson's Research that supports efforts to find a cure for this disease. The symptoms of Parkinson's disease include tremors, bradykinesia, muscle rigidity, as well as impairing speech, writing, and posture [9]. These effects result from the death of the dopaminergic (DA) neurons in the brain that are responsible for controlling movement [10,11]. Similar to Alzheimer's disease, Parkinson's disease itself does not directly cause death, but instead its symptoms can lead to fatal complications [12]. Men are more likely to be diagnosed than women with Parkinson's. While most people are diagnosed with Parkinson's after the age of 50, a subset of patients suffer from early onset Parkinson's [13].

One of the most common therapeutic approaches replaces the dopamine that would normally be secreted by DA neurons, using a precursor molecule called L-DOPA [12]. Continued treatment with L-DOPA leads to dyskinesias (distorted movements), and thus long treatment options for Parkinson's disease must be investigated [14]. Researchers have investigated ways of promoting survival of DA neurons through the delivery of neurotrophic factors as a means of developing an effective strategy for long-term treatment of Parkinson's disease [15]. Specifically, glial-derived neurotrophic factor (GDNF) promotes the survival of DA neurons in animal models of Parkinson's disease as well as the differentiation of embryonic DA neurons grown in culture [16–18]. An ongoing clinical trial by Medgenesis Therapeutix delivers GDNF into the brains of patients suffering from Parkinson's using a novel convection–based technology. Once in the brain, GDNF promotes survival of DA neurons, reducing the effects of Parkinson's disease on motor impairment [19]. Proper delivery of appropriate concentrations of biologically active

GDNF into the correct region of the brain is necessary for achieving functional improvement in motor skills [20]. However, accomplishing this goal remains a challenge due to various issues, including the presence of the blood-brain barrier and the loss of GDNF due to diffusion into surrounding tissue. Stem cell therapy also could be used to replace the neurons that have died due to this disease. A clinical trial implanting fetal-derived neural tissue into the patients suffering from Parkinson's disease was conducted in the late 1980s with mixed results [21]. Current efforts are focused on translating stem cell-derived therapies, including the generation of replacement DA neurons from pluripotent stem cells, into the clinic as soon as 2017 [22].

2.3 HUNTINGTON'S DISEASE

The exact function of the huntingtin protein remains unknown. However, what is known is that a specific mutation in the gene encoding this protein leads to the development of Huntington's disease, making it a genetic disorder [23]. The huntingtin protein is quite large and the mutated version contains an additional 36 glutamine residues, making it even larger. This autosomal dominant mutation affects nerve cells leading to their death in the striatum. The symptoms of Huntington's disease include emotional issues such as depression and anxiety, loss of cognitive function, and physical symptoms, like loss of coordination, which are similar to those experienced by people suffering from Parkinson's disease [24]. Symptoms get progressively worse over time with most patients dying 15–25 years after the disease begins to manifest. The average age at onset is 40 years old. Current treatments only focus on treating the symptoms—the main drug prescribed for Huntington's disease is tetrabenazine, a dopamine-depleting agent that treats the physical symptoms of the disease [24]. Some groups have explored cell therapy as a way to replace the dying nerve cells associated with Huntington's disease [25]. In particular, the California Institute for Regenerative Medicine is currently funding a number of projects that develop different cell therapies to treat this disease. More information on these studies can be found at their fact sheet (https://www.cirm.ca.gov/our-progress/disease-information/huntingtons-disease-fact-sheet). Interestingly, stem cells derived from patients suffering from Huntington's disease serve as a tool to study the basic biology of this disease, which could provide greater insight into how to find successfully a long-term cure for this disease [26]. Another method for potentially curing a genetic disorder like Huntington's disease is to use gene-editing tools to correct the defective gene in the genome and thus prevent the disease from manifesting [27]. Such gene-editing tools will be discussed in Chapter 8.

2.4 AMYOTROPHIC LATERAL SCLEROSIS

ALS, also known as Lou Gehrig's disease, causes rapid, progressive degeneration of the nervous system [28]. This disease gained significant attention worldwide in 2015, as people all over the world performed ice bucket challenges shared on social media to raise awareness of ALS [29]. The term sclerosis refers to a hardening of tissues with the other terms referring to the location of the muscles affected by

this disease. Specifically, cells found in the brain and spinal cord known as motor neurons begin to die off. These cells control our muscles and their death results in weakened muscles that eventually leads to death. The disease progresses rapidly with an average survival time of 3 years and no effective treatments exist currently. While the disease can strike at any time, average age at onset is in the late 40s, with environmental factors, such as serving in the military, increasing the risk of catching this disease [30].

The majority of the reported cases of ALS have no genetic basis with about 10% occurring due to familial inheritance caused by mutations in genes like superoxide dismutase 1 (SOD1) [31]. While it might be possible to use gene therapy to correct these mutations, it still only represents a small fraction of the total patient population. Currently, the only available treatment for ALS consists of the drug Riluzole, which improves the patient's ability to breathe and can extend their lifespan by 2–3 months [32]. However, it is expensive and its efficacy has been debated. In addition, attempts to develop more effective drugs for treating this disorder have not been successful [33]. Thus, many groups have explored the use of stem cell therapies as a way to replace the motor neurons lost to ALS [34]. Other research groups use patient-derived stem cell lines to screen potential drug candidates for efficacy as well [31]. Thus, further development of these stem cell technologies provides potential avenues for finding a long-term cure for ALS.

2.5 MULTIPLE SCLEROSIS

A combination of genetic and environmental factors cause multiple sclerosis, an autoimmune disorder where the body's immune system attacks the myelin sheaths that surround the nerves [35]. Myelin, a protein expressed in the cells that protect and insulate nerves, contributes to the fatty region of the nervous system referred to as white matter [36]. The typical age of onset is around the age of 34, but it can afflict individuals from a large range of ages. This disease can be difficult to diagnose as the symptoms can appear intermittently. These symptoms include fatigue, numbness, issues with vison, and bladder problems [37]. Canada has the highest rate of people suffering from multiple sclerosis in the world, suggesting that its environment plays a strong component in triggering the onset of multiple sclerosis. The most accurate way to confirm if a person has multiple sclerosis is to use magnetic resonance imaging (MRI) to detect lesions present in the nervous system [38]. Similar to ALS, no effective treatment exists for curing multiple sclerosis. The currently available treatment consists of intravenous infusions of the monoclonal antibody natalizumab [37]. However, this treatment costs ~$60,000 per year, creating a significant burden both financially and in terms of the delivery of this treatment. Stem cell therapy offers hope for treating multiple sclerosis with potential therapeutic avenues such as modulating the immune system to prevent attacks on the nervous system, as well as the possibility of designing a cell therapy to replace the cells of the nervous system damaged by this disease [39].