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Review

Embryonic stem cell therapy applications for autoimmune, cardiovascular, and neurological diseases: A review

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Abstract: Parkinson's disease, type 1 diabetes, and coronary artery disease are some of the few difficult diseases to control. As a result, there has been pressure in the scientific community to develop new technologies and techniques that can treat, or ultimately cure these life-threatening diseases. One such scientific advancement in bridging the gap is the use of stem cell therapy. In recent years, stem cell therapy has gained the spotlight in becoming a possible intervention for combating chronic diseases due to their unique ability to differentiate into almost any cell line. More precisely, embryonic stem cell therapy may hold the potential for becoming the ideal treatment for a multitude of diseases as embryonic stem cells are not limited in their ability to differentiate like their counterpart adult stem cells. Although there has been controversy around the usage of embryonic stem cells, there has been found a great deal of potential within the usage of these cells to treat a multitude of life-threatening diseases. In this article, we will break down the categories of diseases in which embryonic stem cell therapy can be applied into: autoimmune, neurological, and cardiovascular with three diseases relating to each category. Our aim is to provide a comprehensive review on the advantages of embryonic stem cells (ESCs) that can solve current obstacles and push advances towards stem cell therapies in the field for the most common diseases.

Keywords: embryonic stem cells; stem cell therapy; autoimmune disease; neurological disease; cardiovascular disease

1. Introduction

ESCs have the ability to differentiate into the three-germ layer of endodermal, mesodermal, and ectodermal cell types depending on its growth and extracellular matrix factor [1]. These factors influence the epigenetic modulation of significant gene expression for the ESC to differentiate into a specific germline [2]. Without relying on donors, a supply of a cell type is readily available for research, drug discovery, and transplantation therapies for heart disease, Parkinson's disease, juvenile-onset diabetes, and other chronic diseases [3].

Stem cells are located inside of a specific niche, the origin of how a stem cell receives its genetic ability to reproduce itself multiple times in self-renewal, or to change into the former cell of the niche location. In the case of ESCs, most are derived from the blastocyst before it plants itself against the placenta. In doing this, the stem cell retains its viability without harming the organism being generated on the inside. However, it was later found that certain neonatal mass contains some stem cell niches within the umbilical cord and fetal blood after birth. Regardless, the niche itself is a potential form of multipotency, as in ESCs, provided with the proper protein structure and cell media influence, can change into several forms of tissue. This methodology proves to be substantial, knowing the various germ layers generate every physiological system when utilized together.

Early research about ESCs first originated when one clonal teratocarcinoma line showed differentiation to other cell lines in vitro [4]. After interest of differentiation of different germline from one cell, the first ESCs were discovered in mice in 1981 [5]. Stem cells in general were becoming promising towards cell transplantation and therapeutic aims to treat diseases such as marrow aplasia and leukemia [6]. Moving on from rodents towards testing on primates, in 1995, ESCs were successfully derived from primates [7]. ESCs were able to differentiate into the three-germ layer and proliferate for eleven months in its undifferentiated state [8]. In 1998, human embryonic stem cells (hESCs) were successfully isolated from human blastocysts [9]. It was not until a couple of years later, in 2002, HESCs were able to derive into other germ layers [10].

ESC therapies are becoming promising treatments for different types of diseases. This review focuses on the advances and obstacles of ESC therapy for preventative medicine and treatment of autoimmune, neurological, and cardiovascular diseases. In particular, a section of the article will examine cardiovascular diseases such as myocardial infarction, coronary heart disease, and stroke that use ESC therapies to replace limited amounts of required cells that the body stops producing. The second section of the article will look at the advancements and obstacles of using ESC therapy towards autoimmune diseases that will help the body's defense system. Autoimmune diseases will include Type 1 Diabetes, Multiple Sclerosis, and Lupus. The third section examines a review of neurological diseases such as Parkinson's disease, Alzheimer's disease, and Cerebral Palsy using ESC therapy to replace or regulate release of cells within the neurological system. The last section of this review paper will examine germ layer properties: controlling differentiation, layer types, and stem cell culturing techniques.

2. Cardiovascular diseases

The heart and the circulatory system, also known as the cardiovascular system, make up the network that transports blood to the body's tissue. It took thousands of years for philosophers to discover the basic principles of how the heart functions as a pump for our body rather than an open-ended system. The Ancient Greeks, including Hippocrates and Galen, viewed the cardiovascular system as two comprising distinct networks of arteries and veins. Galen claimed that the liver produced blood that was then radially distributed to the body, whereas air was absorbed from the lungs into the pulmonary veins and carried by arteries to the various tissues of the body [11]. Harvey's ligations and measurements, Malpighi's microscope, Hale's blood- pressure tube, Ludwig's kymograph, Marey's sphygmograph, and the Einthoven string galvanometer did more to contribute to cardiovascular research than nineteen centuries of unsubstantial speculations. The watch, the stethoscope, and the tonometer established the modern approach to a scientific diagnosis of disorders of the cardiovascular system [12].

Cardiovascular disorders are associated with abnormal blood flow and restriction of blood to the cardiac muscle. These complications can lead to myocardial infarctions, coronary heart disease, and strokes. Current treatments include heart valve replacement surgery, heart transplant, and bypass surgery [13]. However, complications can arise from procedures that are invasive to the heart such as: infection, coronary arteriopathy, blood clots that can lead to heart attack, stroke, or lung problems [14]. Despite recent advancements in treatments for such diseases, heart disease has a yearly mortality rate of approximately 610,000 people in the United States [15]. A new advancing treatment in the cardiology field is the use of ESC therapy for the replacement of damaged tissue in the heart. This section of the article will examine advancements and obstacles of ESC therapy regarding several cardiovascular diseases (CVD).

2.1. Myocardial infarction

Myocardial infarction is caused by the sudden restriction of blood supply and nutrients to tissues. The result is a lack of oxygen and glucose needed for cellular metabolism which keeps the tissues alive [16]. The adult human heart has a limited regenerative response to injury such that the loss or dysfunction of cardiomyocytes results in reduced pump function, often culminating in heart failure, life-threatening arrhythmias, and sudden death [17]. Stem cell transplantation therapy has been used to repair damaged heart tissue for more than a decade and studies have provided profound evidence that the transplantation of cells can help with the regeneration of new tissue on the injured area by enhancing mechanical and biochemically support and therefore restoring the heart muscle functions (Figure 1) [18].

One study investigated the effects of intramyocardial injection of mouse adipose-derived stem cells (mASC) in combination with mouse endothelial cells (mEC) on left ventricular (LV) function and focused on the generation of pericardial fat in mice that were diagnosed with acute myocardial infarction (AMI) [18]. AMI rat models were generated by ligating the left anterior coronary artery, and assigning them to four groups: control (n = 10), mASC (n = 10), mEC (n = 10) and mASC+mEC (n = 10), each rat received 1×10^6 cells around three infarcted areas. Jong et al. [18] used positive Y-Chromosomes staining to verify cell engraftment in specified regions. Fat staining was studied with lipid fixation in paraffin embedded section. The development of new blood vessels was

assessed by staining thin sections with antibodies for smooth muscle alpha actin (α-SMA). Results showed that myocardial infarct size significantly decreased in mASC+mED group compared to control and mEC group. α-SMA was counted in the posterior wall, border, and infarcted zone. There was a noticeable increase in the number of α- SMA- positive vessels found in mASC+mEC group when compared to the control. This indicated that the mASC+mEC injection promotes local angiogenesis in the infarcted zone, resulting in increased LV contractile function supported by PET and echocardiography analysis.

Current treatments fail to address the underlying scarring and cell loss, which is one of the leading causes of heart failure after infarction. Therapeutic angiogenesis using cellular transplantation is a promising strategy to increase blood flow in patients with severe ischemic heart disease. In several studies related to endothelial progenitor cells (EPCs) derived from patients that are then used for autologous transplantation therapy have been shown to foster the formation of arterial collaterals and promote the regeneration of ischemic tissues. However, difficulties in obtaining sufficient amounts of adult EPCs from patients may limit autologous stem cell therapy [19].

Zongjin Jong et al. [19] developed a novel two-step differentiation process with serum free culture system, to increase endothelial differentiation efficiency. First, hESCs were cultured with differentiation medium for 12 days to induce embryos body (EB). Next, EB's were embedded into collagen 1 and subcultured for an additional three days. To assess if human embryonic stem cell-derived endothelial cells (hESC-ECs) were capable of forming functional blood vessels in vivo, Matrigel plug assay was observed for a two-week period. It was noted that several of the hESC-EC derived vessels formed conduits that contained blood flow from day 12 to day 60. To understand the therapeutic potential of hESC-ECs for treatment of CVD, adult mice were injected with 1×10^6 hESC-ECs. Results demonstrated that the transplantation of cells into the infarcted area made a significant improvement in cardiac function for two weeks. However, this was not sustained beyond for weeks. Also, importantly noted was the abundance of decay in bioluminescence suggesting acute donor cell death. The use of adult cells is attractive because of their immune-compatible nature, ease of isolation, limited differentiation potential, and capacity to proliferate rapidly. However, the low potential for cardiac differentiation or integration with host cells limits the benefit of these cells.

On the other hand, ESCs can differentiate into relatively large numbers of early stage cardiomyocytes that functionally integrate with host heart cells [20]. A recent study examined the difference between infarcted mouse hearts when injected with mouse ESC-derived cardiac progenitor cells (CPCs) and when injected with saline (control). One month after injection into the infarcted region of the murine myocardium, results demonstrated that the CPCs engrafted and differentiated into cardiomyocytes, formed gap junctions as well as contributed to neovascularization in the infarcted area. One of the major concerns in using ESCs or their differentiated progeny for cell-based interventions is the risk of neoplastic tumor or teratoma formation due to undifferentiated ESCs. However, no teratoma formation was observed following cell transplantation. It was also pointed out that there was a superior systolic and diastolic performance of the CPC-treated compared to untreated hearts. Results suggest that the narrow differentiation potential of CPCs could be safe for cell based therapies. These findings are consistent with previous reports in demonstrating that transplantation of ESC-derived cells leads to the in vivo cardiac improvement following myocardial infarction [20]. Oren Jong et al. [21] investigated the capacity of hESCs and their cardiomyocyte derivatives (hESC-CMs) to engraft and improve myocardial performance in a rat chronic infarction model. The presence of human cells within the rat hearts was evaluated using PCR-based

deoxyribonucleic acid (DNA) amplification of the α--satellite region of the human chromosome 17. Immunostainings for cardiac-specific markers demonstrated that not only that the in vivo cardiac environment did not enhance hESC cardiomyogenesis, but also that it resulted in almost no differentiation into the cardiac lineage in all hearts studied. It was also noted that in 6 of 10 healthy and in 3 of 6 infracted hearts, injection of undifferentiated hESCs resulted in the formation of teratoma structures. The formation of teratoma structures may indicate the need for a more established procedure to differentiate cells.

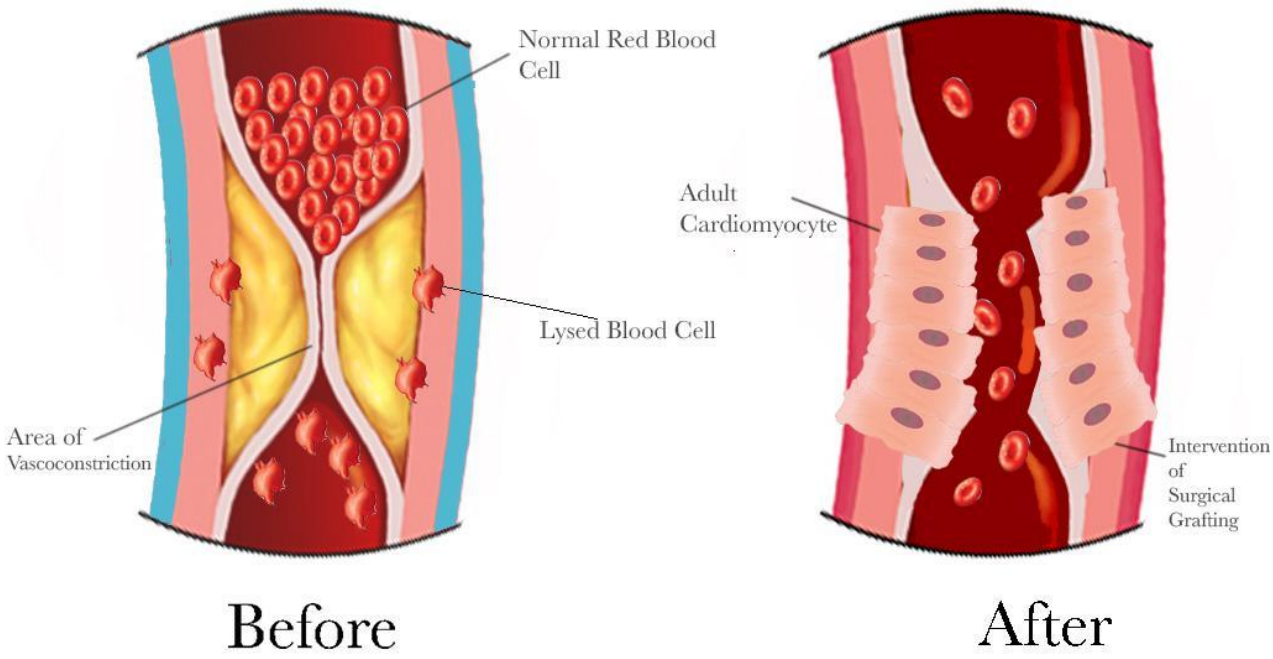

Figure 1. Above is a demonstration of myocardial infarction, where a clot is shown. As the tissue breaks down, blood cells become damaged or lysed since the blockage occurs within heart ventricles. The tissue continues to experience vasoconstriction, causing stress on the muscles. Fortunately, cell signaling releases agents into the area, and with Adult Cardiomyocytes (ACMs) present regenerate cardiac tissue. Due to their ability to recognize the field, ACMs are able to generate into necessary tissues in order to rebuild the ventricles. This is typically done through intervention of cardiomyocytes treated for grafting.

2.2. Coronary heart disease

Coronary artery disease (CAD) is the most common form of heart disease found in adults. The deposition of cholesterol in coronary arteries leads to narrowing which reduces the blood supply to the heart [22]. Cardiac performance after myocardial infarction is compromised by ventricular remodeling, which represents one of the leading causes of late infarct-related chronic heart failure and death [23]. Coronary-artery bypass grafting (CABG) was introduced in 1968 and became the standard protocol for patients with coronary artery disease [24]. Another attractive approach to treat

CAD is to use a balloon expandable coronary-artery stent. These devices hold coronary vessels open at sites that have been dilated. However, long-term outcomes don't show as many positive results as do standard balloon angioplasty [25]. Research has shown that inflammation plays a key role in CAD and atherosclerosis, which is a buildup of fats in and on the artery walls [26]. Various inflammatory cell types like macrophages, neutrophils, and lymphocytes play crucial roles in the destabilization and subsequent rupture of an atherosclerotic plaque, ultimately resulting in atherothrombosis [27]. A more recent advancement is the use of cell transplantation to direct areas of the infarcted heart. One study treated 18 consecutive patients with chronic myocardial infarction by the intracoronary transplantation of autologous bone marrow mononuclear cells and compared them with to a control group which did not undergo cell therapy. Results showed that the transplantation group's infarct size was reduced by 30% and global left ventricular ejection fraction (15%) and the infarction wall movement velocity increased significantly (57%) [23]. Whereas the control group had no significant changes. The clinical significance of this novel approach is that remodeling after infarction may be enhanced or even stopped by this procedure.

Endothelial progenitor cells (EPCs) play a fundamental role in not only blood vessel development but vascular repair [28]. EPCs assist angiogenesis and have been linked to ischemia-related disorders, including coronary artery disease (CAD). hESC-ECs can be applied to different cardiovascular research and disease treatment areas. Endothelial cells sense Gram-negative bacteria through receptors (PRR) Toll-like receptor (TLR)-4 and nucleotide-binding oligomerization domain-containing Protein (NOD)-1. These pathways are critical regarding detecting infection, but TLR4 is also associated with vascular inflammation. Imperial College London compared TLR4 and NOD1 responses in hESC-EC with those of endothelial cells derived from other stem cells and with human umbilical vein endothelial cells (HUVEC). They found that hESC-EC do not respond to TLR agonists however they do express all of the necessary intracellular signaling to ascend an inflammatory response, offering a potential therapeutic advantage [29]. Omar El-Mounayri et al. looked into deriving and characterizing functional coronary-like VSMCs from hESCs using serum-free cardiac-directed differentiation [30]. Functional characterizations including contractile responses and integration into new vessel formation in vivo support the opportunity to employ these cells for disease modeling, drug screening, and applications in cell-based therapies for regenerative medicine. The ability to generate hESC-derived functional human coronary-like VSMCs in serum-free conditions looks promising for regenerative therapies; however, more research must be conducted.

2.3. Stroke

Ischemic strokes occur when an artery to the brain is blocked. The brain depends on its arteries to bring blood from the heart and lungs. If the artery remains blocked for more than a few minutes, brain cells may begin to die [31]. The timing of treatment is critical because the longer a patient waits to get treatment, the more likely it is that the risks of treatment will outweigh the benefits [32]. Stroke is a leading cause of serious, long-term disability in the United States [33]. Because the time frame in which treatment to the patient must be administered is very crucial, there is limited treatment; however, the use of stem cell therapy has shown to have benefits in the healing process after ischemic stroke. At the core of the infarcted cavity, the affected cells die rapidly by necrosis.

Stem cell therapy for ischemic stroke focuses on restoring neural elements but also supporting structures such as blood vessels [34]. There have been many clinical studies done on mice that

demonstrates that the transplantation of stem cells can improve functional recovery. For example, several studies using mesenchymal stem cell (MSCs) have demonstrated a functional recovery after middle cerebral artery occlusion in rats and improved long-term functional outcome. However, the integration of transplanted cells into the ischemic brain, with the replacement of dead cells, is an unlikely mechanism of repair. Most studies have shown the survival of very few transplanted cells following neuron transplantation, despite evidence of significant functional recovery [34]. Jieli Chen et al. tested the intravenous infusion of human marrow stromal cells (hMSCs) with a nitric oxide donor (NONOate) to see if it would enhance angiogenesis, neurogenesis, and neurological functional recovery after stroke in rats compared to each therapy done on its own. Functional tests and immunohistochemistry showed that NONOate plus hMSCs in combination significantly induced functional recovery, it also significantly increased vessel perimeter and endothelial cell proliferation compared with hMSCs or NONOate alone as a treatment [35].

hESCs can ultimately offer a virtually unlimited source of neural cells for structural repair in cardiac-neurological disorders, such as stroke [36]. A recent study investigated the differentiation of neural precursors from hESCs and how transplantation of cells would benefit mice after ischemic stroke. The differentiation of the neural precursors was measured using immunohistochemistry and an adhesive removal test was used to examine functional improvement after stroke. Results showed that after 11 days, hESCs expressed at least one neural marker, meaning successful differentiation into neural precursors. Transplantation of these cells improved regenerative activities and sensory function [37]. However, one major concern that remained during experimental period was the formation of tumors. No teratoma formation was observed, which can be an indication of the safety level of this procedure. Although current experiments look promising, more testing must be done.

3. Autoimmune diseases

The immune system protects the bodies' internal system from illnesses and viruses by attacking and neutralizing foreign microorganisms. However, this defense system can also cause problems to arise when immune cells begin destroying healthy cells, tissues, and organs by recognizing healthy cells as the source of an illness or infection. Such a response from the organism's immune system can result in one of the hundreds of autoimmune diseases, from Addison's disease to the rare Granulomatosis with Polyangiitis (GPA). The pathogenesis and etiology of many autoimmune diseases are yet to be discovered. Environmental and genetics factors have been investigated in their role in the development of autoimmune diseases and have been found they seem to interact in the development of autoimmunities [38–42]. Over the past thirty years, incidences and the prevalence to autoimmune diseases has increased [43]. As a result, there has been a great amount of work dedicated in discovering a curative therapy for patients diagnosed with autoimmune diseases.

Current medical interventions for treating autoimmune diseases include: immunosuppressive medication [44], hormone replacement therapy [45], blood transfusion [46], anti-inflammatory medication [47], pain medication [48], and physical therapy [49]. However, many of these interventions can cause further complications to arise such as an increased risk of serious infection and certain types of cancers. With advancements in the scientific field, new therapies to treat autoimmune disease are developing such as costimulation blockade, regulatory T cell therapy, antigen-specific immunotherapy, manipulation of the interleukin-2 pathway [50], and embryonic

stem cell therapy. This section of the article will examine advancements and obstacles of ESC therapy in relation to three autoimmune diseases: type 1 diabetes, multiple sclerosis, and lupus.

3.1. Type 1 diabetes

Type 1 diabetes is an autoimmune disease in which an organism's immune system destroys its' insulin producing beta cells in the islets of Langerhans [51]. As a result, one who is diagnosed with type 1 diabetes has abnormal blood sugar levels, and if untreated, can be life-threatening. Since the etiology continues to be unknown, treatments currently aim at achieving normoglycemia. To achieve this, several techniques have been used such as islet transplantation, pancreas transplantation, and insulin injection therapy. Unfortunately, transplantation requires the usage of immunosuppressants, which can be toxic to the transplanted islets and surroundings organs. This technique is also very limited due to the shortage of islet donors [52].

A recent and developing technique to combat type 1 diabetes is the usage of ESCs (Figure 2) due to their unique ability to differentiate into many cell types, presumably also islet cell types [53–55]. The first protocols designed to promote differentiation into insulin-secreting cells specifically were developed for mESCs due to the fact they preceded that of the first hESC lines. In a study conducted by Naujok et al. the group was able to differentiate mouse ESCs into insulin-producing cells and the cells were able to release insulin in response to glucose stimuli and to normalize the blood glucose levels in diabetic mice when transplanted into those mice [56]. Furthermore, it was shown that pancreatic endoderm derived from human embryonic stem (hES) cells efficiently generated glucose-responsive endocrine cells after implantation into mice [57]. The data provides definitive evidence that hES cells are competent to generate glucose-responsive, however, it also showed the risk of developing tumors [57].

A similar study using hESCs revealed SCID/NOD mice treated with hESC-dervied pancreatic IPCs corrected hyperglycemia for more than 8 weeks and tumor formation was not evident in this time span [58]. D'Amour et al. demonstrated that in vitro culture conditions that mimicked embryonic pancreas development resulted in differentiation of hESCs into definitive endoderm and subsequently into insulin-producing β-like cells [59–60]. In another study, Gregory L. Szot et al. demonstrated that immunotherapies that target T cell costimulatory pathways block the rejection of xenogeneic human embryonic-stem-cell-derived pancreatic endoderm (hESC-PE) in mice. The therapy allowed for long-term development of hESC-PE into islet-like structures capable of producing human insulin and maintaining normoglycemia [61]. Further studies have also revealed generating insulin cells from embryonic stem cells using different procedures [62–66].

Due to breakthroughs that have been established throughout the years, using ESC therapy to treat type 1 diabetes has shown a great of potential. However, using ESC therapy currently faces many hurdles before reaching the clinics. As mentioned earlier, one of the risks of using embryonic stem cells as a form of therapy for type 1 diabetes is the development of tumors [57]. Although researchers have been capable of differentiating insulin-producing cells from ESCs, none of the end-stage results met the requirements for surrogate beta-cells. Aside from that hurdle, there is the risk of spontaneous differentiation into an undesired cell type.

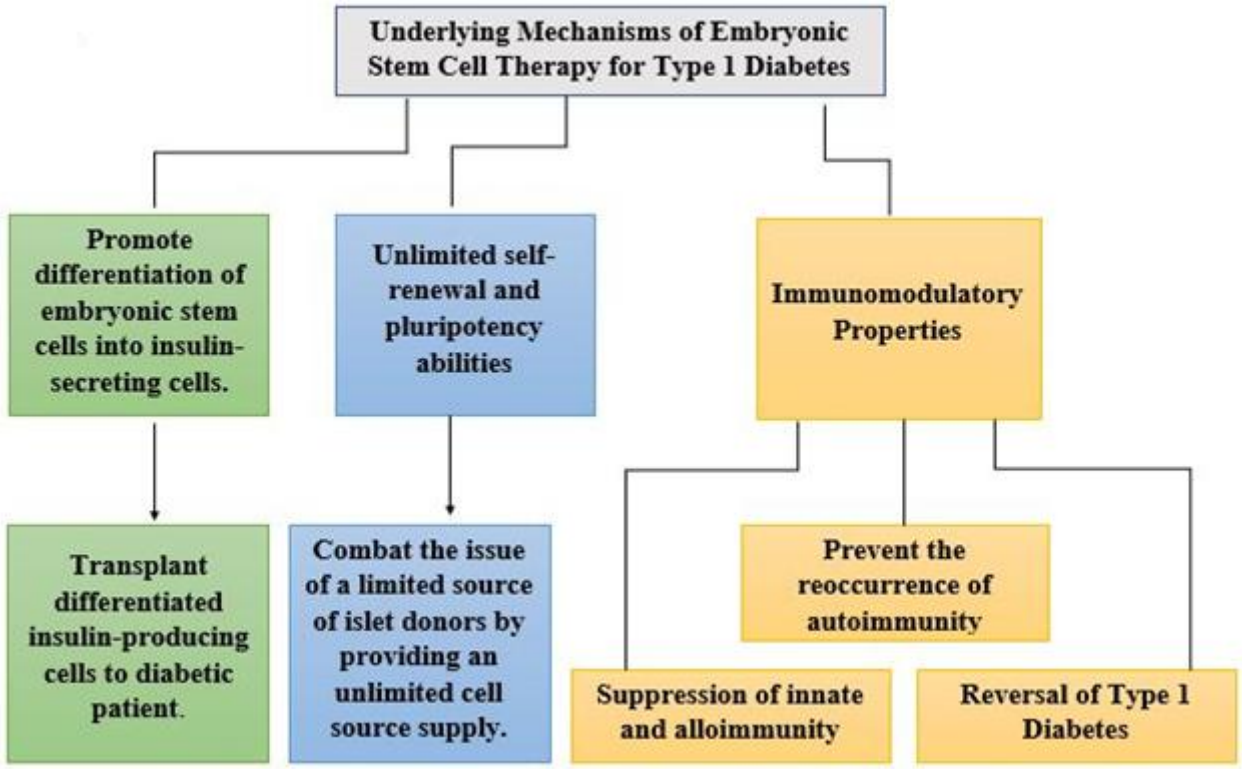

Figure 2. Underlying mechanisms of embryonic stem cell therapy for combating type 1 diabetes.

3.2. Multiple sclerosis

Multiple sclerosis (MS) is a common autoimmune disease in which the immune system eats away the sheaths covering nerve cells in the brain and spinal cord. The result is nerve damage that disrupts communication between the brain and the body. Although the pathogenesis is still unknown, the current consensus is that MS is triggered by environmental agents acting in genetically susceptible people [67]. The disease is diagnosed based on clinical findings and supporting evidence from ancillary tests, such as magnetic resonance imaging (MRI) of the brain and examination of the cerebrospinal fluid (CSF) [68]. Current treatments include interferon-β1a intramuscular (Avonex), interferon-β1a subcutaneous (Rebif), interferon-β1b subcutaneous (Betaseron/Extavia), glatiramer acetate (Copaxone), natalizumab (Tysabri), fingolimod (Gilenya), teriflunomide (Aubagio), and mitoxantrone (Novantrone). In addition, many clinical trials are being conducted to assess the safety and efficacy of various experimental agents in patients with multiple sclerosis, including alemtuzumab, dimethyl fumarate, laquinimod, rituximab, daclizumab, and cladribine [69]. However, depending on the medication, some potential side-effects include: flu-like symptoms with headache, fever, chills, fatigue, vomiting, depression, suicidal ideation, or deterioration of psychiatric disorders, elevated liver enzymes, the development of an opportunistic infection of oligodendrocytes by JC virus known as progressive multifocal leukoencephalopathy (PML), hair thinning, and mildly increased hepatic enzymes [68]. Due to the adverse side effects, new therapies continue to be developed in hopes of treating or ultimately curing multiple sclerosis.

One emerging therapy is the usage of ESCs due to their capacity to give rise to different cell types such as oligodendrocytes (myelin-producing cells) [70–73]. In one study, hESCs-derived early

multipotent neural precursors (NPs) were transplanted into the brain ventricles of mice induced with experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Results demonstrated that transplanted hESC-derived NPs significantly reduced the clinical signs of EAE [74]. Another group evaluated the therapeutic potential of neural stem cells(NSCs) derived from ESCs by two different neural differentiation protocols and found that the NSCs did not have a major beneficial impact in an EAE model; however, ESC-derived NSCs exerted differential immunosuppressive effects, which are important properties of NSCs [75]. In two different rodent models of induced demyelination, it was demonstrated that ESCs differentiated into glial cells and re-ensheathed demyelinated axons [71,76]. One of the more recent studies using ESCs for treating multiple sclerosis has been conducted on a human, a 42-year-old male. The study found that hESC therapy was effective and safe on the patient [77]. The male exhibited an improvement in balance, cognitive skills, fiber tracts, b/L central semi-ovals and sub-cortical regions of front parietal fiber, D9-D10 levels as well as a diminishing of prior symptoms he had (weakness and fatigue). After the study, the patient recovered without any problems.

As mentioned before, researchers have demonstrated the usage of ESCs could be a 'doubled-edged sword' due to spontaneous differentiation and formation of teratomas [78,79]. In addition, there is also the hurdle of immune rejection by the recipient's immune system; however, there are studies indicating ESCs and ES cell-derived NSCs display characteristics of immunotolerance [75,80,81]. Although the studies mentioned have shown a great deal of therapeutic potential for treating those with multiple sclerosis, more studies are still needed to prove the long-term safety and effectiveness for treating people with multiple sclerosis.

3.3. Lupus

Lupus is a chronic autoimmune disease in which the immune system attacks its own tissues which results in long-term inflammation and damage to several organs of the body. There have been found to be four main forms of lupus: neonatal [82], discoid [83], drug-induced [84], and systemic lupus erythematosus (SLE) [85]. For each of the different forms of lupus, symptoms can vary greatly, which can make determining the correct diagnosis of lupus a complication. Furthermore, the etiology of lupus is yet to be discovered. As of now, lupus is thought to be the result of genetic and environmental factors. SLE, commonly referred to as "lupus", is the most common form of lupus and will be the focus of this review. Current treatments have adverse side-effects and are not curative [86,87], but they include the usage of antimalarial agents, topicals, and immunosuppressants. Because of unsuccessful therapies, researchers have begun looking at cell-based therapies as a form of treatment.

Although there are relatively few studies related to the usage of ESCs for treating lupus, there have been two studies in which results were similar and displayed promising results for combating lupus. In the first, Kimbrel and her group, using a mice model, were the first to demonstrate the therapeutic efficacy of hESC-MSCs for lupus [88]. Results showed lupus-prone mice injected with hESC-MSCs exhibited a marked increase in survival. Similarly, in another study conducted by Thiel et al. the group showed hESC-MSC treatment can prolong survival in a lupus-prone mouse model and delay SLE disease progression [89]. Even though both studies resulted in great discoveries, there still needs to be more investigation done to prove the long-term safety and effectiveness of ESCs for lupus therapy.

4. Neurological diseases

Exploration of the nervous system can be dated back to the second century A.D. by Galen, who was the first to document parts of the nervous system, such as the superior and inferior cervical ganglia and also hypothesized that nerves were used as pipes to flow "animal spirits" between organs [90]. Due to the limitation of early microscopy and embryology, little could be done to prove or answer ideas about the nervous system while many continued to be under the influences of Galen's concept of spirits [90]. It was not until the early nineteenth century when scientists developed technology to understand the nervous system, showing the electrical nature of nervous conduction by Du Bois Reymond in 1843 [91]. Later in the nineteenth century, increasing discoveries were made regarding the nervous system such as the two types of involuntary nerves (autonomic and reflex) by Langley and synapses for chemical transmission signals by Sir Henry Hallett Dale [92].

As technology advances, people are able to accurately understand the nervous system, paving the way to enhance research regarding neurological diseases, while developing more successful treatments. Neurological disorders are disease related to the nerves, spine and brain. Such diseases include Parkinson's Disease, Alzheimer's Disease, and Cerebral Palsy. Existing methods to treat neurological diseases include ancient therapeutic acupuncture [93] and intake of clinically approved drugs [94]. A new advancing treatment toward the neurological field is the use of ESC therapy. This section of the article will examine advancements and obstacles of ESC therapy regarding neurological diseases.

4.1. Parkinson's disease

One of many neurological diseases is Parkinson's Disease, a degenerative disease that affects motor control due to dopaminergic neuron death in the substantia nigra [95]. Dopamine is an important chemical for transferring information from one neuron to another neuron until it reaches the muscle, affecting the control of a person's movement [96]. Due to late detection of deficiency in the neurotransmitter dopamine, disease-modifying therapies may be ineffective [97]. Currently, there is no exact cause of the deterioration of the cells, but seems to be more common in men compared to women, usually affecting ages over fifty, and caused from a combination of environmental and genetic factors. While there is no cure for Parkinson's disease, levodopa is a medication administered to patients to help make more dopamine. With levodopa in the brain, patients can at least try to cope with symptoms that include slurred speech, a loss of balance, and a slowness of movement [98]. Previous treatments include deep brain stimulation, showing effective motor controls but gray understanding of the mechanism behind it [99]. A recent advancing treatment is the use of ESC therapy to restore dopaminergic dysfunction and intervene Parkinson's progression by neural transplantation from limitless hESC-derived homogeneous dopaminergic progenitors and neurons [100].

Neuroscientists have successfully derived dopamine-producing neurons from mouse ESCs and implanted it within the mouse's brain leading to an average of forty-percent more improvement than rats who received sham surgeries [101]. The beginning steps of recovery for Parkinson's patients are regular amounts of dopamine release and neuronal activity. Using optogenetics to examine real time neurochemical and electrophysical properties of implanted mesencephalic dopaminergic neurons derived from human embryonic stem cells, neuronal activity and dopamine release can be observed which can lead to the identification of a mechanism for

Parkinson recovery. Past studies have suggested that a full behavioral recovery from Parkinson's disease must involve functional integration of grafted dopamine neurons. A current obstacle for the process of functional neuronal integration is the lack of methods associated with neuronal graft function. However, the application of optogenetics done in this study allows for the reversible functional manipulation of specific neurons [102]. Another examination for the impact of implantation of hESCs differentiating into dopaminergic neurons is the use of screening phytochemicals with dopaminergic neurogenesis-boosting potentials, examining voltage-gated ion channels, dopamine release, neuron function, and dopamine receptors agonists bromocriptine and 7-hydroxy-2-(dipropylamino) tetralin (7-OH-DPAT). This study resulted in identifying ginsenoside Rb1 as the most potent phytochemical for the purpose of influencing differentiation and upregulation of neurotrophin expression [103]. Aside from rodents, studies have been conducted on other animals such as monkeys.

To restore midbrain dopaminergic neurons, adult green male monkeys, otherwise known as chlorocebus sabaeus, are given doses of dopaminergic neurons derived from hESCs. The results showed extended neurite outgrowths, dopaminergic-induced phenotype, and expression of synaptic markers [104]. Usage of genetically modified ESCs have shown to survive at least 6 weeks [105] and for derived dopamine neurons had no neural progenitors overgrowth in rodents and primates. The study conducted on these rodents with Parkinson's disease confirmed the survival of dopamine neurons from ESCs. With neurites that innervate the striatum, behavioral deficits in the rodents were reversed. Future studies have the goal of reconstructing the basal ganglia circuitry to have more permanent effect on the disease [106]. Using ESC therapy towards Parkinson's disease shows promising results such as delayed neuronal death. However, obstacles are simultaneously seen such as increased susceptibility to mitochondrial inhibition, oxidative stress, and proteasome inhibition [107]. While challenges are present in the use of embryonic stem cell deriving into dopamine neurons, results have shown potential in recovery for Parkinson's disease in the brain.

4.2. Alzheimer's disease

Of the many neurological diseases that exist, Alzheimer's disease is one of the most recognizable because it the fourth leading cause of death in people over the age of sixty-five [108]. Alzheimer's disease is a brain disorder characterized by degeneration of nerve cells, the presence of amyloid plaques, and the presence of neurofibrillary tangles that ultimately leads to memory loss and cognitive decline (Figure 3). In addition, Alzheimer's disease progressively worsens with age and contributes to slow-wave activity in the brain [109]. Even in the early stages of Alzheimer's disease, patients struggle to carry out everyday tasks and struggle to maintain concentration [110]. Because the exact cause of Alzheimer's disease continues to be argued among health professionals, effective treatments are difficult to come by and many theories still exist about its causes [111]. For example, some clinical researchers attribute the impairment of memory to the synaptic dysfunction caused by assemblies of the amyloid β protein. Thus, leading to alterations of the hippocampal synaptic efficiency and then neuronal degeneration [112]. Neuronal degeneration that ultimately alters the neurons when compared to individuals who do not have Alzheimer's disease. Due to the decrease of dendritic branching and the weakening of synapses, neurons begin to lose the unique structure responsible for the transfer of information. Elements of the structure such as the axon, myelin sheath, and membrane break down causing the alteration of structure of the cell body. Another approach

simply cites genetics as the cause of Alzheimer's disease [113]. For patients with mild to moderate Alzheimer's disease, treatment usually includes the use of cholinesterase inhibitors. Before Alzheimer's disease can interrupt or kill neurons, cholinesterase inhibitors slow down this process. After treatment, patients tend to improve with regard to cognition and have better long term-results when compared to patients that have not received treatment [114]. In an attempt to find more treatment options, clinical trials that focus on aspects of the disease such as inflammation, neurotrophic function, and processing of proteins have begun [115].

Despite its large impact, treatments for Alzheimer's disease have not been able to effectively care for the disease and the need for new therapies has become evident. One of the main reasons is that the acquisition of live neurons from patients proves to be challenging and thus limits the understanding of the disease's pathogenesis. As a result, hypotheses such as the amyloid cascade hypothesis that memory loss is caused by increased levels of Aβ peptides cannot be fully confirmed [116]. While cholinesterase inhibitors are one of the most known ways to treat Alzheimer's disease and increase cholinergic function, the treatment is temporary and fails to cure the disease. Many times its potential to reduce the decline of cognitive abilities is overshadowed by its frequent side effects. These include nausea, vomiting, and weight loss [117]. Due to the unique ability of embryonic cells to divide indefinitely and differentiate, research involving embryonic stem cell therapy has proven to be developmental with neurodegenerative diseases such as Alzheimer's disease.

Many findings suggest that ESCs may perhaps replace lost cell populations [116]. Since no treatment is currently curative, studies have attempted and successfully generated neurons and glial cells from ESCs [117]. One of the studies conducted included eight patients with mild Alzheimer's disease and included implanting modified fibroblasts in order to express nerve growth factor (NGF) in the forebrain. This is due to the fact that NGF stimulates cholinergic function and helps prevent the cholinergic neuron loss that characterizes Alzheimer's disease. Without the use of these ESCs, the size and polarity of NGF prevents it from crossing the blood-brain barrier and therefore NGF cannot be peripherally administered into the brain. In addition, the immobility of fibroblasts after transplantation can be replaced by these genetically modified stem cells with high migratory capacity. After twenty-two months, the results of this study indicated an increase in 18-fluorodeoxyglucose and growth responses in the brain [118]. Since genetically modified ESCs can carry new genes after being transplanted into the brain, they have the potential to replace fibroblasts that often become immobile after transplantation [119]. Thus, suggesting that new methods may soon be discovered to replace damaged or lost brain cells which coincide with Alzheimer's disease [120]. However, in order for ESC therapy to advance, more research into clinical application must be applied to address obstacles that are currently present. These include, but are not limited to, the understanding of structural reorganization and recovery processes after transplantation [117] The unpredictable interactions that may result from transplantation must also be considered. With the development of these studies, more can be learned about the effects of ESC therapy which will allow future studies to begin from a larger base of information.

Another approach that has been taken includes induced pluripotent stem cells (iPSCs) created with mouse fibroblasts to mimic the properties of ESCs. While there is still much debate surrounding the true similarity between iPSCs and ESCs, much of iPSC technology is based upon the ability of ESC therapy to generate traits remarkably similar to that of naturally existing cells [121]. In a recent study, the fibroblasts of two patients with familial Alzheimer's disease, two patients with sporadic Alzheimer's disease, and two control individuals were reprogrammed into iPSCs. Neurons obtained

from differentiated cultures and later purified by fluorescence-activated cell sorting were used to examine protein levels and check for any significant relationships. Neurons cultured with fetal messenger RNA samples formed functional synaptic contacts. A very important result was that the phenotypes relevant to Alzheimer's disease could be observed thus characterizing the importance of ESC therapy [122]. This pathway to relevant phenotypes is just one of the many benefits of ESCs because it can take years or even decades for Alzheimer's disease to even be identified. An earlier detection of the disease is a promising step towards a treatment with more permanent effects. In accordance with this study, a model of familial Alzheimer's disease has been created with iPSC technology. With human neurons containing a presenilin 1 mutation and presenilin 2 mutation, an increase in the forty-two amino acid form β-amyloid (Aβ) peptides by living human neurons has been noted. This supports the amyloid cascade hypothesis that states the deposition of the amyloid- β peptide as a contributing factor in Alzheimer's disease. Thus, helping to analyze the veracity of current theories and develop new techniques that are less invasive to the nervous system [123].

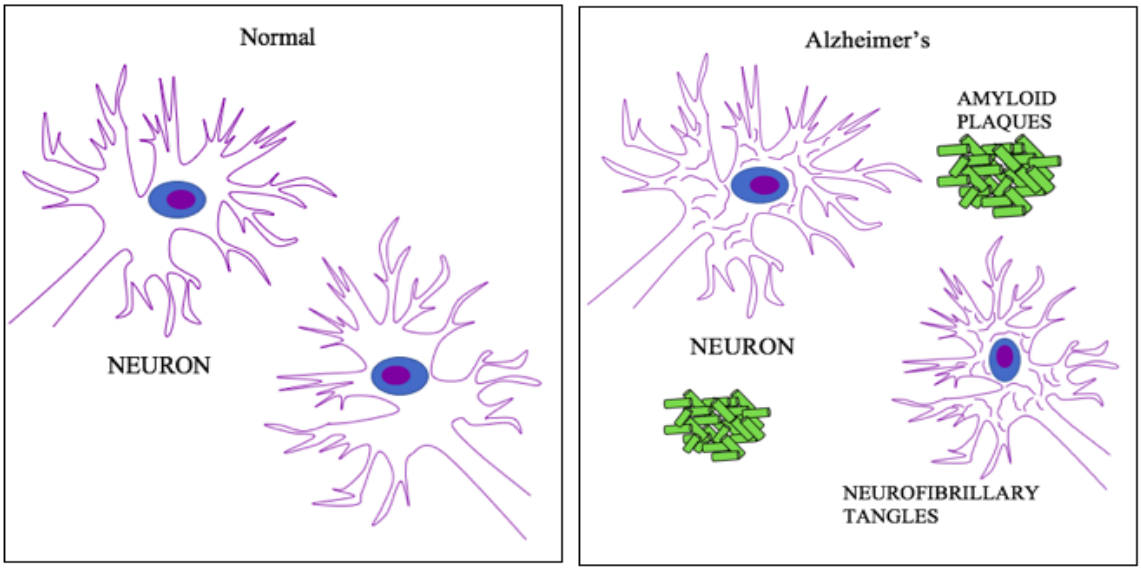

Figure 3. On the left side, neurons with fully functioning structure are displayed while the right side displays neurons with presence of neurofibrillary tangles and amyloid plaques caused by Alzheimer's disease.

4.3. Cerebral palsy

One of the most known neurological diseases is cerebral palsy because it is attributed as the most common physical disability found in children [124]. Cerebral palsy is used to refer to a group of neurological disorders appearing in infancy and early childhood that affect body movement, muscle coordination, and balance. Thus, can be characterized by muscle stiffness and asymmetric gross motor function that ultimately prevent or alter motor development. As a result of these limitations, children with cerebral palsy struggle with social and behavioral situations. However, unlike other neurological diseases that worsen with time, cerebral palsy tends to maintain itself consistent when comparing disabilities and condition over time [125]. Because cerebral palsy is a

term used to describe numerous disorders, it has several different causes, clinical types, and developmental pathologies [126]. Prior to birth, antenatal causes of cerebral palsy include congenital malformations, vascular complications such as middle cerebral artery occlusion, or maternal infections that occur early on during pregnancy. During delivery, perinatal causes of cerebral palsy include cord prolapse or obstructed labor because of the hypoxia that results. Although less common, post-neonatally acquired cerebral palsy is caused by infections by meningitis and malaria or injuries resulting from vehicular or near-drowning accidents [127]. Much of the treatment used today focuses on improving the overall life of children with cerebral palsy by improving developmental abilities, functionality, and social interactions [126]. Just as there are several types of cerebral palsy, there are also several treatments. One of these treatments is neurodevelopmental treatment which is used to control the sensorimotor components such as muscle tone, sensation, and memory, yet long-term effects have not been consistent enough to support it. Common treatments include physical therapy and medications. Surgical treatments such as a selective dorsal rhizotomy involve cutting dorsal rootlets from the spinal cord to eliminate spasticity (Figure 4). By doing so, the amount of abnormal signals sent from muscles to spinal cord are reduced [128]. The inability of treatment to fully improve the wellbeing of patients has led to an interest in ESC therapy. Using embryonic stem cell therapy compared to current or previous types of methods to treat cerebral palsy open the doors to using donor less cells that are restore the body back to its original state instead of getting rid or cutting parts of the neural system.

Due to the unique ability of ESCs to differentiate into any cell type, ESC therapy has proved to be promising in patients with cerebral palsy. Research performed on rat models with transplanted ESCs have demonstrated improved learning ability. The release of neurotrophic factors and improvement in locomotor mechanisms has led to an extensive study on patients with cerebral palsy [129]. hESC therapy was received by ninety-one patients with cerebral palsy and tested for hypersensitivity reactions. The study was divided into four treatment phases (T1, T2, T3, T4) by gap phases in relation to time. The doses of hESCs in each phase were relatively the same, but differed in gap phases. With the help of the caudal route, injections, and eye drops, hESCs were able to reach the spinal fluid and thus help regenerate the spinal cord. All the patients also received physiotherapy and rehabilitation. Results for this study indicated that patients demonstrated improvement in cognitive skills, no serious or harmful events were observed, and improved perfusion in the brain. More specifically, more than ninety-percent of all age groups in this study demonstrated improvement [130] Furthermore, another study has used hESCs for cortical visual impairment in forty children with cerebral palsy. After dividing the study into treatment phases separated by gap phases that allowed cells to multiply, patients' levels of visual impairment were monitored. The results demonstrated that thirty-nine out of forty patients showed improvement in vision thus showing the positive effects of hESC therapy. Because hESC therapy has not been experimented extensively, more studies are needed to support hESC therapy and its ability to prevent degeneration [131]. Although there are very few studies related to the use of hESC therapy, it is expected to eventually develop to treat disorders like cerebral palsy [132]. Studies have shown progress, such as past studies indicating that the differentiation of hESCs have helped gain progenitor cells at necessary sites such as those caused by brain injuries [133].

While hESC therapy is a new field with the potential for more research, it has its own number of setbacks in contributing to neurological diseases such as cerebral palsy. As an area with a limited amount of research, hESC therapy does not possess a cell protocol or process to generate

transplantable cells. Without a set approach to generate desired cell types, the risk of contamination grows. Even after intracerebral transplantation, the cells implanted must be able to survive and function. When cells are grafted, dividing hESCs with chromosomal instability may cause chromosomal abnormalities that cause it to outgrow other cells during transplantation. These imbalances ultimately lead to the formation of tumors. Finally, the differentiation of cells may trigger unwanted immune reactions preceded by graft rejection. Inflammation or rejection of the cells implanted threaten the ability of ESC therapy and become serious risk factors.

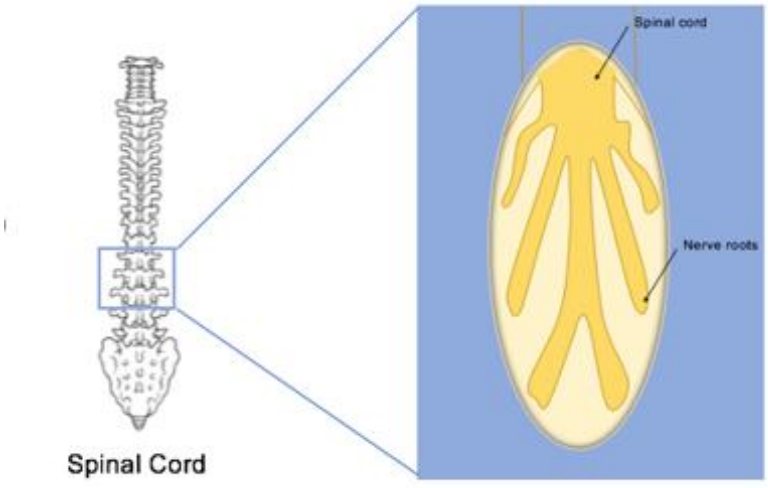

Figure 4. Surgical treatment for cerebral palsy known as selective dorsal rhizotomy involves targeting and cutting rootlets located in the spinal cord.

5. Germ layer properties: controlling differentiation

Fundamental cells are capable of producing on one another, with the singular purpose of recreating itself for homeostasis. This is only a basic element of the cell, but in most cases, especially the subject here within, ESCs are capable of several layers of differentiation [134]. Unique from other cell lines, ESCs have been specially developed to recreate several tissue layers in order to animate an organism inside of the fetus. Modern technology has found that ESCs can be removed from the embryo itself, given the rich pluripotency of the cell. For a cell to exhibit its multiple patterns of differentiation, three germ layers that embody the gastrula must be present: ectoderm, mesoderm, and endoderm. All of these are unique to its own, bringing rise to what researchers and scientists can use as an advantage for recreating tissues. Although there is some risk associated with these unique properties, the embryonic tissue created can still be formed without damaging any organism created when harvested for ESCs [135,136].

5.1. Layer types

Several cells begin to overwhelm the exterior of the blastocyst during the development of the embryo. The ESCs that make up this embryo needs to mature in order to develop multiple tissue types. This eventually forms a gastrula, an inner cell mass comprised of the three germ layers [137].

To clarify, the ESCs used in therapies are not developing another embryo. Rather, the ESCs utilized in certain therapies are modeled off of this system to differentiate into other tissue types [138]. These layers match and specify which cells are required for that cell lineage (Figure 5). Ectoderm is responsible for creating the central nervous system, including the peripheral nervous system, and its surrounding layers. It forms the other sensory parts connected to the nervous system, including the eye and other sensory epithelia. Second to it is the mesoderm, the layer that generates the majority of the organs. Here, the mesoderm also provides structure to the embryo by initializing the skeletal system, the growth of muscles and fibers, and imperative systems like the urinary and cardiovascular system. Inconclusive to the generation of all said tissues is the endoderm (sometimes termed entoderm), which focuses on structuring the epithelial lining of interior organs, thyroid glands associated with a few other organs of the endocrine system, and the pancreas.

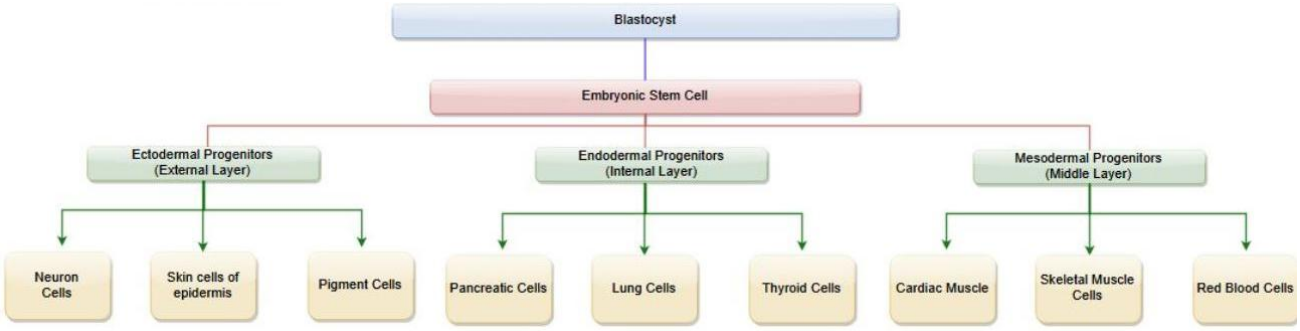

Figure 5. Differentiation of embryonic stem cells. ESCs are able to differentiate into all the cell types of the three germ layers: ectoderm (such as neuron cells, skill cells, and pigments cells), endoderm (such as pancreatic cells, lung cells, and thyroid cells), and mesoderm (such as cardiac muscle cells, skeletal muscle cells, and red blood cells).

All of these build on one another to form the pluripotency of the ESCs to develop multiple tissue types [139,140]. Not much research has been shown as to how these layers effectively work within one another to determine the embryonic structure. Some speculation suggests that the mesoderm is the most advanced, where embryology has seen that only a germ monolayer was present. The mesoderm made up for most of this, until further progress showed the ectoderm and endoderm slowly evolved to complete the blastocyst. Much of this was demonstrated in a study to suggest vertebrate embryos initially were not as developed as primates were. A mesoderm at time fulfilled the systems to generate organisms like zebrafish [141]. Yet, a certain conclusion is needed to find whether or not the mesoderm influences the entire cell. It can be claimed that the lineage of each germ layer was made official, genetics has yet to prove otherwise.

There is some debate over using induced pluripotent stem cells (iPSCs) over embryonic stem cells. Both share techniques demonstrated for drug delivery experiments, serve as the cornerstone of regenerative medicine, and can both change cell type. For one advantage, iPSCs are more accessible as they are acquired from skin or blood cells to be reprogrammed into a pluripotent like state. These cells revolutionized the idea of creating more stem cells, rather than acquiring embryonic stem cells from neonatal tissue. However, a common issue found in iPSCs is the ability to use regenerative medicine techniques performed in rats over to humans. Multiple trials have shown slow progression,

where not many iPSCs were able to stay controlled. Some cannot define their new genotype and fail to be reprogrammed except for eye and some skin tissue. Though ESCs are not as accessible, the ones harvested are immortal and maintain pluripotency outside of the embryo. Certain conditions are required to emulate the environment of an embryo, but this is done within the following section.

5.2. Stem cell culturing techniques

Limited experience with stem cell culturing started without growth factors or any influences, undifferentiated stem cells can still have potential for subculturing for future therapeutic uses. In some cases, a feeder layer is required for the stem cells in order to establish a path of differentiation. A feeder layer is made up of fibroblasts to prevent the cells from dividing. The layer is sometimes treated with a certain chemical, Mitomycin-C or occasionally T-proteins, in order to prevent further replication. Like cell cultures, the stem cells are grown in the appropriate cell culture dish, given time to grow undifferentiated until confluent, and soon are exposed to new agents that react to the cells appropriately (Figure 6). Although this method is appropriate, it does not always consider the three-dimensional factors that are necessary since the culture works in a two-dimensional environment.

As mentioned before, ESCs mimic the environment typical of the embryo developed in an organism. A recreation of the stem cell niche inside of the Extracellular Matrix can influence a healthy stem cell culture, increased growth, and control over the differentiation [142]. Cell lineage of what tissue will be created is another issue. Once ESCs are created, most of them require specific agents or signals. In traditional cell culturing, media includes the supplements required for supporting the cells. Examples of agents that serve to change the cell type include Bone Marrow Protein, Fibroblast Growth Factor, and amino acids to alter the genotype of what will be developed. The two most studied are Oct4, a genome that directs pluripotency, and Bone Morphogenic protein (BMP) which encourages stem cell growth. Both of these are typically found in stem cell niches, though much has been discussed on BMP. There are several variations of BMP, but it is imperative as its former derivation (Bone Marrow itself) is capable of regenerating many tissue layers, including nerve, bone, blood, and fat tissues.

These factors have been studied to be developed for cellular reprogramming, a tissue engineering technique that takes the genetic nucleus of one cell to be redesigned in another [143]. Currently, ESCs serve as a role model for reprograming for its pluripotency and ability to react appropriately to specific differentiation. This is commonly done on adult skin cells, where the somatic nuclear transfer of an ESC is transferred over to fibroblasts. This in turn creates a larger ‘pool’ of both ESCs and fibroblasts, but more importantly overwhelms the environment with more stem cells [144]. Much of this has been proven as the karyotype of fibroblasts are quite manageable to manipulate. But a current issue is preventing karyotype abnormalities. Teratomas are redeveloped after gene expression is altered, but in some cases teracarcinomas are provoked in the culture. Yet this is much more common with ESCs at multiple cultures, which is why primary passaging is encouraged. Fibroblasts can extend to several passages, where reprogramming increases expansion and confluency of multiple cultures. The only foreseeable issues with reprogrammed fibroblasts from ESCs are longevity, as some patients treated may need a biopsy to determine the genetic control of the fibroblasts over time.

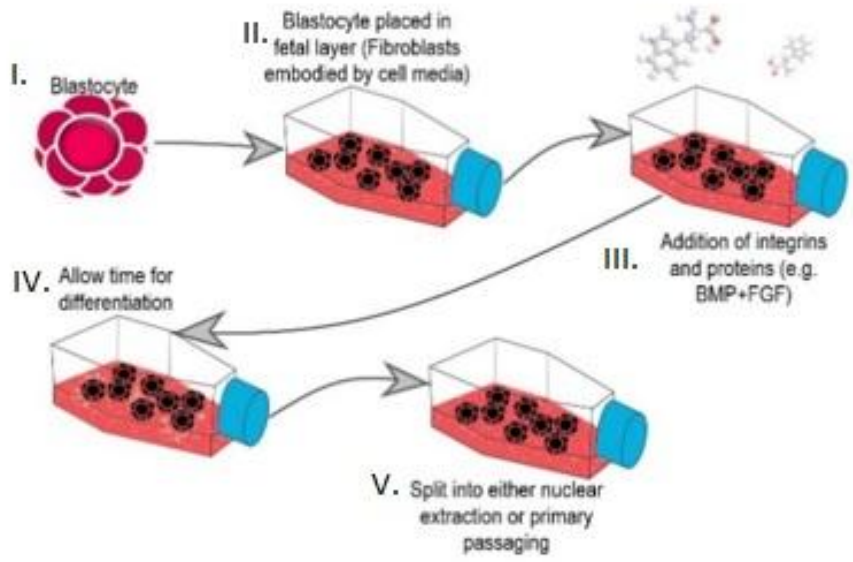

Figure 6. The process of Embryonic Stem Cell Culturing is as follows. (I) The Embryonic Stem Cell comes undifferentiated. Pluripotency is to be controlled as long as culturing occurs, as free radicals or proteins can alter this delicate cell. (II) Culturing begins as the blastocysts are placed above a fibroblast layer (in red) within a culturing flask. Media is also included. (III) Proteins and integrins are added in addition to maintaining pluripotency or to change the cell type. Proteins above shown are general, as any protein could be used. (IV) Time must be given to allow the Embryonic Stem Cells to adapt to the new environment. Typically, this lasts between 1–2 weeks for growth and further culturing. (V) The ESCs are ready for one of two common therapies: nuclear extraction or primary passaging. Extraction of the nucleus to a basic cell type (i.e. fibroblasts) to reproduce more ESCs or the like, whereas primary passaging is focused on increasing the stem cell count for the specific phenotype it is to be cultured in.

Today, research investigators have distinguished said factors in a three-dimensional environment. Impact from factors, including extracellular matrix, microenvironment structure, soluble factors, and substrates can all define cell lineage. Biologically, cell signaling places a similar effect on the development of the stem cell growth [145]. Cultures are designed to provide enough space, oxygen, and nutrition, and without the proper matrix such factors cannot be implemented [146,147]. Synonymously, a few factors depend on the ECM as a basis for controlling stem cells. Integrins can easily bind between each encapsulated cell to provide surface adhesion between the regenerated tissue, including FAK and PI3K [148]. Several other integrins are involved, each varying to the cell type. For all stem cell types, Beta-1 controls the asymmetric and symmetric divisions, as it would for several neuronal cells. The ECM also encourages cells to reshape themselves without damage. In fact, isometric tension creates a mechanosensing technique cells adapt to that regulate cell behavior and prevent irregular cell migration.

What sort of Matrix is debatable for these cells in particular. A common substrate that also acts as a particular extracellular matrix is Matrigel, considered as a ‘gold standard’ for culturing. Matrigel

has exclusive properties that are known to enhance pluripotency in ESCs for several passages. However, Matrigel is unlike other biomatrices. One of the most common aspects that define differentiation biophysically is cytoplasmic reticulum. Especially for ESC cells, the form and shape of the cell controls what cells are possible and organizes multiple tissue layers. For this reason, several other biomatricies are to be considered [149]. ESCs have been found to be more adaptable to Collagen (I and IV), which matches a human microenvironment *in vivo* to organized cellular layers and symmetric cell lines [150].

Some difference is observed when using different substrates and gels, but none are too impactful. In addition to these, some tissue engineers have been able to develop a useful method via bioprinting, where stem cells control their differentiation easily with a specified extruder onto a surface. By utilizing biomatrices, several chambers actuate different stem cell lineages as designated for the development of vascularized tissue that remains animated post-printing. A technique like these is not as common, though companies like Organovo have been successful with stem cell patches for tissue replacement therapies [151].

6. Future directions

While the usage of ESCs for therapeutic uses remains controversial, many animal trials have resulted in promising results for treating autoimmune, cardiovascular, and neurological diseases. In addition, so have the few human clinical trials involving embryonic stem cell therapies. However, it is important to note that human clinical trials often involve a relatively small number of patients and that the span of the study usually is not long enough to access the risk of carcinogenesis. As a result, the human clinical trials results may not be correct in all details when deciding the long-term effectiveness and safety of the therapy in larger populations. For that reason, more clinical trials are needed to assess the long-term efficacy and safety of embryonic stem cell transplantation and for now stem cell therapies should only be utilized as a last resort when conventional therapies are not applicable or have been unsuccessful. Further research on understanding of the mechanisms, proteins, and genes partaking the transformation of hESCs into functional cell types can lead to the discovery of genes that are in the center of controlling tissue differentiation. Along with this, a better understanding of the mechanisms of stem cell signaling and intercellular communication, as well as the ability to counteract the formation of teratomas. Doing so, it can transform the way we can potentially treat type 1 diabetes, Alzheimer's, cardiac infarction, and other autoimmune, neurological, and cardiac diseases.

7. Conclusion

The purpose of this review was to summarize results of studies using embryonic stem cells (ESCs) to combat three cardiovascular, autoimmune, and neurological diseases: myocardial infarction, stroke, coronary heart disease, type 1 diabetes, multiple sclerosis, lupus, Alzheimer's disease, Parkinson's disease, and cerebral palsy (Table 1, Table 2, Table 3) as well as to discuss stem cell culturing techniques. It is evident from the research and results reviewed that embryonic stem cell therapy holds a great deal of potential for treating a multitude of diseases, specifically the ones reviewed in this article. This field of regenerative medicine is of paramount important as it revolves around the proposal of novel therapies for previously terminal conditions. The ability of ESCs to

differentiate into any cell line and to proliferate indefinitely under the right conditions has paved way for studies such as the ones reviewed in this article to be conducted to examine how well ESCs or their differentiated tissues perform physiologic functions in models of human diseases.

Table 1. Summary of Results using ESCs to treat Cardiovascular Diseases.

Differentiated Cell	Results	Reference
Myocardial infarction		
Adipose-Derived Stem Cell	Mice models were injected adipose derived stem cells along with endothelial cells to investigate ventricular function. After 28 days results showed that cells successfully implanted in the region and an increase in pericardial fat which helps in the recovery of heart function.	[18]
Human Embryonic Stem Cell-Derived Endothelial Cells	Differential of hESCs into endothelial cells (hESC-ECs) showed favorable differential without the formation of any teratoma. in vivo testing revealed that hESC-ECs possess the functional vasculogenic ability. But most importantly hESC-ECs significantly improved short-term cardiac function by two weeks.	[19]
Stem Cell-Derived Cardiac Progenitor Cells	CPCs engrafted and differentiated into cardiomyocytes, formed gap junctions as well as contributed to neovascularization in the infarcted area.	[20]
Cardiomyocyte derivatives (hESC-CMs)	The transplantation of hESC-CMs after myocardial infarction results in the formation of stable cardiomyocyte grafts.	[21]
Coronary Heart disease		
Autologous Bone Marrow Cell	Transplantation group's infarct size was reduced by 30% and the global left ventricular ejection fraction was reduced by 15%, it was also observed that the infarction wall movement velocity significantly increased by 57%.	[23]
Embryonic Stem Cell Derived-Endothelial Cells	They found that hESC-EC do not respond to TLR agonists however they do express all of the necessary intracellular signaling to ascend an inflammatory response, offering potential therapeutic advantage.	[18]
Vascular smooth muscle cells	Functional characterizations including contractile responses and integration into new vessel formation in vivo support the opportunity to employ these cells for cell-based therapies.	[19]
Stroke		
hMSCs	NONOate plus hMSCs in combination significantly induced functional recovery, it also significantly increased vessel perimeter and endothelial cell proliferation.	[37]
Neural precursors from human embryonic stem cell	Results showed that after 11 days hESC expressed at least one neural marker, meaning successful differentiation into neural precursors. Transplantation of these cells improved regenerative activities and sensory function.	[35]

Table 2. Summary of Results using ESCs to treat Autoimmune Diseases.

Differentiated Cell	Results	Reference
Type 1 Diabetes		
Insulin-producing cells from mouse embryonic stem cells.	After implantation into diabetic mice these insulin-producing cells produced a time-dependent improvement of the diabetic metabolic state.	[56]
Glucose-responsive endocrine cells derived from human embryonic stem (hES) cells	The insulin-expressing cells generated after engraftment exhibit many properties of functional beta-cells, including expression of critical beta-cell transcription factors, appropriate processing of proinsulin and the presence of mature endocrine secretory granules. Implantation of hES cell-derived pancreatic endoderm protects against streptozotocin-induced hyperglycemia.	[57]
Pancreatic insulin-producing cells (IPCs) differentiated from hESCs	Differentiated pancreatic IPCs derived from hESCs can correct hyperglycemia in SCID/NOD mice for ≥8 weeks.	[58]
hES cell-derived insulin-expressing cells	The hES cell-derived endocrine cells were capable of synthesizing the pancreatic hormones insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin. It was further shown they have an insulin content approaching that of adult islets and release C-peptide in response to multiple secretory stimuli, but only minimally to glucose, similar to fetal β-cells.	[59]
Human embryonic-stem-cell-derived pancreatic endoderm (hESC-PE)	The ESC-PE xenografts were rejected in B6 mice, but blocking the CD28 and CD40L-CD40 costimulatory pathways protected hESC-PE grafts from rejection. for over 90 days. Also, the hESC-PE grafts effectively regulated blood glucose (BG) independently of endogenous insulin-secreting β cells in most STZ-treated NSG mice and did so to a similar extent in NOD-SCID mice.	[61]
Multiple Sclerosis		
ESC-derived neural precursor (NPs) cells.	Transplantation of hESC-derived NPs attenuates the clinical signs of EAE and reduces CNS inflammation and tissue injury.	[74]
NSCs (neural stem cells) derived from embryonic stem cells	Intravenously injected NSCs displayed no significant therapeutic impact on clinical and pathological disease outcomes in mice with experimental autoimmune encephalomyelitis (EAE).	[75]
Neural differentiated mouse embryonic stem cells	Transplanted-derived cells survived and differentiated into astrocytes, oligodendrocytes and neurons, and migrated as far as 8 mm away from the lesion edge. Furthermore, gait analysis demonstrated that transplanted rats showed hindlimb weight support and partial hindlimb coordination.	[76]

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Embryonic Stem Cell-Derived Glial Precursors	Transplantation in a rat model of a human myelin disease demonstrated that the ES cell–derived precursors interact with host neurons and efficiently myelinate axons in brain and spinal cord.	[71]
Human Embryonic Stem Cells (hESCs)	Male who received transplanted exhibited an improvement in balance, cognitive skills, fiber tracts, b/L central semi-ovals and subcortical regions of frontal parietal fiber, D9-D10 levels as well as a diminishing of prior symptoms he had (weakness and fatigue). After the study, the patient recovered without any problems.	[77]
Lupus		
Human embryonic stem cell derived-mesenchymal stem cells (hESC-MSCs)	The administration of hESC-MSCs significantly prolonged the lifespan of BWF1 mice diagnosed with ln (lupus nephritis).	[88]
Human embryonic stem cell (hESC)-derived MSCs	Treatment with hESC-MSCs prevented disease-associated interstitial inflammation, protein cast deposition, and infiltration of CD3(+) lymphocytes in the kidneys. This therapy also led to significant reductions in serum levels of tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6), two inflammatory cytokines associated with SLE. hESC-MSCs also prevented the progression of fatal lupus nephritis (LN) in NZB/W F1 (BWF1) mice.	[89]

Table 3. Summary of Results using ESCs to treat Neurological Diseases.

Differentiated Cell	Results	Reference
Parkinson's Disease		
Dopamine-producing neurons	Rats who had dopamine-producing neurons implanted within their brains showed an average of 40% improved brain activity compared to rats who received sham surgeries.	[101]
Mesencephalic Dopaminergic Neurons	Optogenetics are used to observe neurochemical and electrophysiological properties of mesencephalic dopaminergic neurons showing its dopamine release and graft neuronal activity.	[102]
Midbrain Dopaminergic Neurons	Ginsenoside Rb1 was found to be the most potent phytochemical for the purpose of influencing midbrain dopaminergic neurons differentiation and upregulating neurotrophin expression.	[103]
Dopaminergic Neurons	Dopaminergic neurons derived from human embryonic stem cells were implanted into primate of monkey model of Parkinson's Disease, which exhibited maintenance of dopamine-induced phenotypic, expression of synaptic markers, and extended neurite outgrowths.	[104]

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Genetically Modified Human Embryonic Stem Cell	Grafted human embryonic stem cells expressed tyrosine hydroxylase, relieved symptomatic motor activity, and survived for at least 6 weeks when in the rat's brain,	[105]
Dopamine Neurons	Dopamine neurons derived from human embryonic stem cells have survived in both rodents and primates, showed no teratomas, and no neural progenitors overgrowth.	[106]
Dopaminergic Neurons	Embryonic stem cells from mice in Parkinson's disease model demonstrated delayed neuronal death, but higher susceptibility to oxidative stress, mitochondrial inhibition, and proteasome inhibition.	[107]
Alzheimer's Disease		
Embryonic stem cells	Since embryonic stem cells divide indefinitely and differentiate, it suggests that transplanted stem cells can replace dysfunction cell populations in neurodegenerative diseases.	[116]
Embryonic stem cells	Embryonic stem cells have successfully generated neurons and glial cells while transplantation therapies have been developed.	[117]
Genetically modified embryonic stem cell	Implanted fibroblasts in mild Alzheimer's disease patients were modified to express human nerve growth factor, Thus, resulting in improvement in the cognitive decline rate and no found long-term adverse effects on patients.	[118]
Genetically modified Embryonic stem cell	The ability of modified embryonic stem cells to express genes allows for these to cells to replace fibroblasts become immobile.	[119]
Embryonic stem cells	Manipulations performed on cells allow for certain properties to be resembled and suggest that embryonic stem cell therapy will allow damaged and lost brain cells to be replaced.	[120]
Induced pluripotent stem cells	Induced pluripotent stem cells in mouse made to express characteristics found in embryonic stem cells have the potential to act with great similarity to naturally existing cells.	[121]
Induced pluripotent stem cells	The implantation of iPSCs in fibroblasts has allowed for the phenotypes of patients with Alzheimer's disease to be observed.	[122]
Neuronal cells	Human neurons with presenilin mutations have been used to model familial Alzheimer's disease	[123]
Cerebral Palsy		
Human embryonic neural stem cells	Embryonic stem cells obtained from the temporal lobe cortex and later implanted into adult rats confirmed its beneficial impact on improve locomotor mechanisms.	[129]

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Human embryonic stem cells	After hESC therapy on ninety-one patients with cerebral palsy helped regenerate the spinal cord, results demonstrated the improvement of cognitive skills and perfusion in the brain.	[130]
Neuronal cells	Use of human embryonic stem cells in forty children with cerebral palsy and cortical visual impairment have demonstrated beneficial effects such as improved levels of vision for treatment of cortical visual impairment.	[131]
Human embryonic stem cell derived neurons	Derived neurons implanted into the brain of an experimental rat model survived for five months and raised hope that hESC therapy will become developed enough to treat brain injuries.	[132]
Derived progenitor cells	With the differentiation of human embryonic stem cells in the brain, developments in hESC therapy may eventually lead to treatment for central nervous system disorders.	[133]

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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