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The utility of stem cells for neural regeneration

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Abstract

The use of stem cells in biomedical research is an extremely active area of science. This is because they provide tools that can be used both in vivo and vitro to either replace cells lost in degenerative processes, or to model such diseases to elucidate their underlying mechanisms. This review aims to discuss the use of stem cells in terms of providing regeneration within the nervous system, which is particularly important as neurons of the central nervous system lack the ability to inherently regenerate and repair lost connections. As populations are ageing, incidence of neurodegenerative diseases are increasing, highlighting the need to better understand the regenerative capacity and many uses of stem cells in this field.

Keywords

Stem Cell, Neurodegeneration, Regeneration, Central Nervous System,

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Introduction

The use of stem cells to model neurological diseases in vitro and provide a source of cells for transplantation is an extremely active area of research. Stem cells, due to their immortal nature and ability to differentiate, can provide a pool of relevant cell types for transplantation or in vitro use, allowing researchers to probe the molecular mechanisms underlying neurological disease. This review aims to provide a comprehensive examination of recent literature in the field of neuroregeneration with a focus on the use of stem cell technology in this dynamic field of research.

Many disorders of the nervous system ranging from trauma to neurodegeneration involve the loss of neuronal connections. This highlights the importance of understanding the underlying mechanisms and promoting neural regeneration. The response of the nervous system to injury and the ability of neurons to regenerate, restoring lost neuronal connections, are dependent upon the location of the insult (Gordon, 2016). Nerves within the peripheral nervous system (PNS) can regenerate to some extent following injury due to the supportive growth environment provided by Schwann cells (Bhangra et al., 2016). However, unlike PNS injury, injury to the central nervous system (CNS), including spinal cord injury (SCI), results in the formation of an inhibitory environment. For example, the formation of a glial scar post SCI can result in the inhibition of neurite regeneration due to the release of inhibitory chondroitin sulphate proteoglycans (CSPGs) from reactive astrocytes and inhibitory molecules found on myelin debris from damaged neurons (Rolls et al., 2009; Xu et al., 2015; Yiu and He, 2006).

The inability of neurons to regenerate in the glial scar or to reinnervate severe lesions in the PNS results in functional deficits that may have a significant impact on the quality of life for an individual. Not only is such loss of neural connectivity heavily implicated in the clinical outcome of these types of physical trauma, it is also common to many disorders of the CNS including stroke or neurodegenerative diseases such Alzheimer's disease, Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS; Adalbert and Coleman, 2013; Martinez-Morales et al., 2013). For this reason, the promotion of neural regeneration is an important avenue for medical research and designing therapeutics that promote neuronal regeneration could help alleviate the symptoms of a large number of nervous system disorders. There are a number of strategies under development, including the use of cell-based approaches and the development of stem cell technologies to replace damaged or lost tissues.

Stem cells have the ability to self-renew and differentiate into mature, specialised subtypes, providing a useful tool in the study of neuronal cell loss (Martello and Smith, 2014; Martinez-Morales et al., 2013; Tabar and Studer, 2014). The ability of stem cells to differentiate into neural subtypes not only is applicable to cellular replacement therapies but can also provide novel insights into the biomolecular mechanisms that underpin neural degeneration through forming the basis of in vitro cell-based models (Avior et al., 2016; Trounson and DeWitt, 2016).

Classification of stem cells is based on their developmental potential (Figure 1). Pluripotent stem cells give rise to specialised cell types from each of the three germ layers, whereas multipotent

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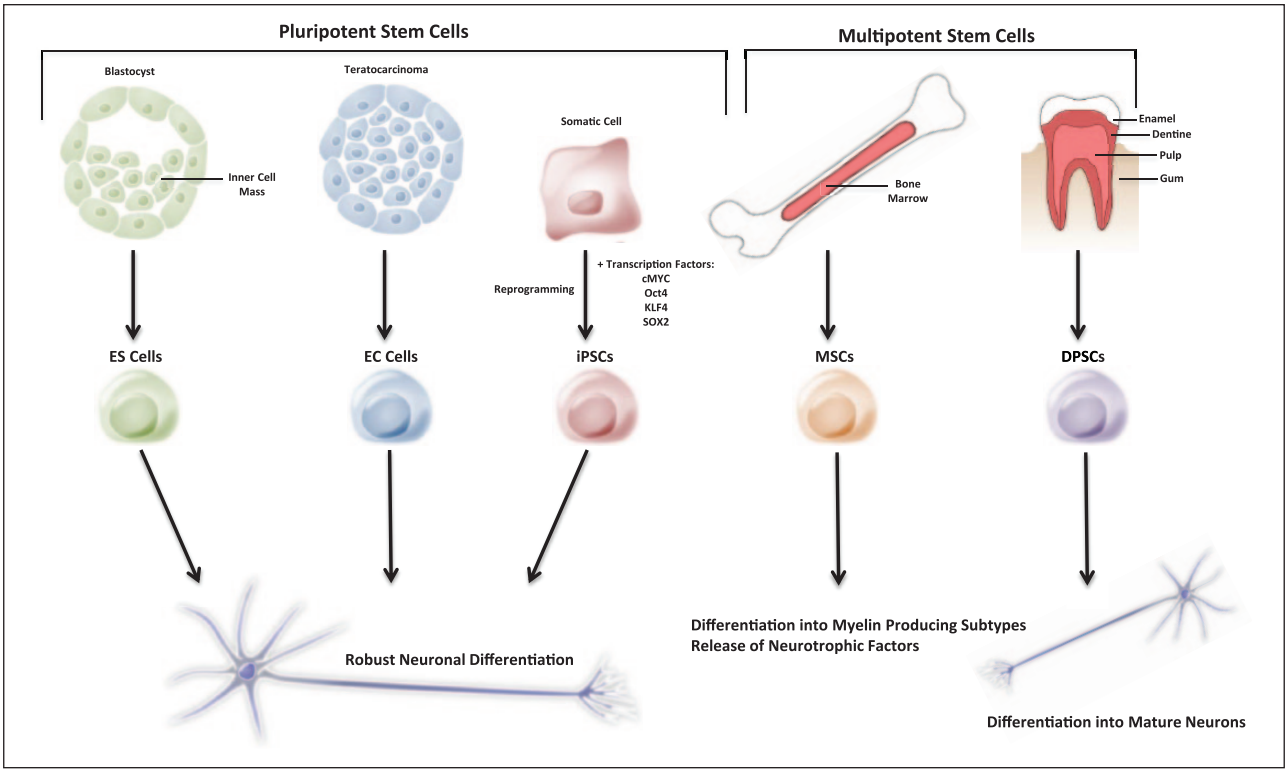

Figure 1. Types of stem cell commonly used to study and treat neurological deficits. Pluripotent stem cells are unique in their ability to self-renew and differentiate into specialised cellular subtypes. They include human embryonic stem (ES) cells derived from the inner cell mass of the developing blastocyst, embryonal carcinoma (EC) cells, the malignant counterpart of the ES cell derived from teratocarcinomas and induced pluripotent stem cells (iPSCs), which are formed from the reprogramming of somatic cell types using a cocktail of transcription factors. Pluripotent stem cells undergo robust neural differentiation when stimulated with morphogens to produce a pool of neural derivatives that form the basis of in vitro models to study neural degenerative or regenerative responses. In contrast, multipotent stem cells are more lineage restricted and some type may provide a supportive role. For example, multipotent mesenchymal stem cells (MSCs) from the bone marrow can differentiate into myelin-producing glial subtypes that if transplanted can provide trophic support to damaged neurons within the nervous system. This strategy can provide a more regenerative microenvironment to encourage endogenous neural regeneration. Alternative sources of cells with beneficial properties include dental pulp stem cells (DPSCs) that can successfully differentiate to form mature neurons and have been tested during transplantation into animal models of SCI and enabling functional recovery.

stem cells can differentiate into more restricted lineages (Martinez-Morales et al., 2013). Pluripotent stem cells include human embryonic stem (ES) cells that are derived from the inner core of the developing blastocyst, and their malignant counterparts, embryonal carcinoma (EC) cells, derived from teratoma tumours (Przyborski et al., 2004). More recently developed induced pluripotent stem cell (iPSC) technology involves the reprogramming of adult somatic cells into an undifferentiated, pluripotent cell with the capacity to differentiate into mature cell types (Takahashi et al., 2007; Takahashi and Yamanaka, 2006, 2016). ES and EC cells form the basis of many in vitro models of neural differentiation, development and regeneration (Clarke et al., 2017; Roloff et al., 2015; Wichterle et al., 2002); however, they are rarely used to generate neurons for cellular replacement therapies due to ethical issues in the case of the ES cells and the tumourigenic properties of EC cells (Andrews, 2002). iPSCs are derived from mature adult cells, for example, skin fibroblasts, that can be reprogrammed to become pluripotent and can subsequently be differentiated towards more specialised cells including neural lineages (Sharma, 2016; Trounson and DeWitt, 2016). iPSC-derived neurons provide a unique opportunity for cellular replacement therapies as mature neurons can be obtained from a skin biopsy of a patient (through reprogramming of dermal fibroblasts and subsequent differentiation), producing patient-specific cells for implantation (Sharma, 2016). iPSC technology is also beneficial in the development of patient-specific, in vitro cell-based models of neural regeneration, which can be used for patient-specific drug screening and personalised medicine applications (Avior et al., 2016).

Multipotent stem cells include neural stem cells (NSCs), which are the precursors of mature neurons and glia. There are several examples of where NSCs have successfully been used to replace neurons lost, including application to treat ischemic stroke (Hicks et al., 2013; Mack, 2011). Human adult dental pulp stem cells (DPSCs) are also multipotent stem cells that are thought to originate from migrating cranial neural crest cells and are located within the perivascular niche of dental pulp (Martinez-Morales et al., 2013). DPSCs have the ability to differentiate into mature neurons, transplantation of which has resulted in functional recovery in animal models of SCI and stroke (Leong et al., 2012; Nosrat et al., 2001). Mesenchymal stem cells (MSCs) originate from tissues that include bone marrow, adipose tissue and peripheral and umbilical cord blood (Martinez-Morales

et al., 2013). Promising clinical reports have suggested that MSCs may improve functional recovery in ischemic stroke patients, with the beneficial properties of MSCs thought to include paracrine and autocrine action in damaged tissues, through the secretion of growth factors and anti-inflammatory cytokines (Chamberlain et al., 2007; Han et al., 2014; Murphy et al., 2013; Teixeira et al., 2013; Ylöstalo et al., 2012).

In this review, we address the role of both pluripotent and multipotent stem cells in two main aspects of neural regeneration. First, we consider the development of stem cell–based models of neural systems to further our understanding of regenerative mechanisms and to screen potential molecules for their ability to induce neural regeneration. Second, we provide an overview of examples for the latest stem cell–based cellular replacement therapies and their ability to recover functionality in disorders of the nervous system.

Stem cell–based in vitro models of neural regeneration

Modelling neural differentiation of stem cells in vitro is an important tool that can be used to investigate the molecular pathways involved in neural regeneration and also screen molecules that may be used to modulate regenerative responses. Induction of neural differentiation by stem cells is most often achieved by activating signalling pathways involved in the development of the nervous system (Schwartz et al., 2008). For example, the retinoic acid (RA) pathway is involved in patterning and differentiation of the nervous system, and stimulation of retinoid signalling is often used in vitro to promote neuronal differentiation of stem cells (Clarke et al., 2014; Janesick et al., 2015; Maden, 2002; Maden and Hind, 2003; Roloff et al., 2015). Similarly, inhibition of glycogen synthase kinase-3 (GSK-3) by the molecule TWS119 involved in Wnt signalling and induction of fibroblast growth factor (FGF) signalling are also commonly used to modulate neural differentiation of stem cells (Clarke et al., 2014; Schugar et al., 2007; Schwartz et al., 2008).

New technologies are being developed to continually improve our ability to control neural differentiation by stem cells. RA is a metabolite of vitamin A that ultimately results in the induction of gene expression of retinoid-inducible genes, some of which are responsible for neuronal development (Clarke et al., 2014; Janesick et al., 2015; Maden, 2007; Maden and Hind, 2003). The vitamin A derivative, *all-trans* retinoic acid (ATRA), is commonly used to induce neuronal differentiation in vitro. However, its use in vitro is limited due to its ability to readily break down when exposed to light and heat, thus leading to variable and inconsistent induction of development. Synthetic forms of the molecule such as EC23 have been designed, which contains a non-isomerisable linker unit, resulting in a more stable and potent compound (Christie et al., 2008; Clemens et al., 2013). EC23 has been shown to induce neuronal differentiation and neurite outgrowth in a robust and reproducible manner, and to a significantly greater extent than the naturally occurring ATRA in a stem cell model of neuritogenesis (Clarke et al., 2017). Some of the most common in vitro models of neurite outgrowth are based on pluripotent stem cells differentiated with retinoids to produce functional neurons (Clarke et al., 2017; Hayman et al., 2004; Pewsey et al., 2010; Przyborski, 2001; Przyborski et al., 2003, 2004; Tegenge et al., 2011). Human pluripotent stem cell–derived models such as these can be advantageous over other non-human neurite outgrowth models, as they are more applicable to studying human physiology and disease. In addition, primary cell cultures derived from animal models and immortalised cell lines such as neuroblastoma cells often have a limited capacity for neuritogenesis (Kovalevich and Langford, 2013).

Stem cell–based models of neurite outgrowth are often used to study the molecular processes that inhibit neurite regeneration. Inhibition of neurite outgrowth occurs in nervous system disorders ranging from CNS trauma (Fawcett and Asher, 1999; Niederost et al., 2002; Xu et al., 2015; Yiu and He, 2006) to neurodegenerative diseases (Petratos et al., 2008; Postuma et al., 2000; Takenouchi et al., 2001) and has been implicated in schizophrenia (Miyoshi et al., 2003; Ozeki et al., 2003) and Down's syndrome (Roizen and Patterson, 2003). An example application of an in vitro model to study the process of neurite inhibition is one that occurs following SCI and the formation of the glial scar that prevents the reinnervation of damaged neurons (Rolls et al., 2009; Yiu and He, 2006). Following injury to the spinal cord, astrocytes become reactive and secrete inhibitory molecules including CSPGs which are thought to induce Rho A signalling through a receptor-mediated mechanism, resulting in growth cone collapse and inhibition of neurite outgrowth (Dent et al., 2011; Jeon et al., 2012; Sainath and Gallo, 2015). Further understanding of the downstream signalling cascade that results in the loss of neurite outgrowth is important in the development of novel therapeutic targets to overcome such inhibition and ultimately restore neurite outgrowth. As the activation of Rho A signalling is thought to be the main mechanism responsible for CSPG-mediated neurite inhibition, molecules focused on inhibiting Rho A and its downstream effectors have been used to study stem cell–derived models of neurite outgrowth (Figure 2; Clarke et al., 2017; Lehmann et al., 1999; Lingor et al., 2007; Monnier et al., 2003; Roloff et al. 2015). Inhibition of Rho A through C3 transferase (Gu et al., 2013; Minase et al., 2010; Monnier et al., 2003) or the non-steroidal anti-inflammatory drug ibuprofen (Roloff et al., 2015) has had positive effects on neurite regeneration in vitro. Similarly, the inhibition of Rho A kinase (ROCK), a downstream effector of Rho A, through the use of both the selective ROCK inhibitors, Y-27632 (Clarke et al., 2017; Gopalakrishnan et al., 2008; Monnier et al., 2003) and fasudil (Gopalakrishnan et al., 2008; Lingor et al., 2007), has been shown to significantly enhance neurite regeneration in vitro. Neurite outgrowth models such as those derived from human stem cells are useful assays that can be used to study the effect of manipulating Rho A signalling to overcome an inhibitory stimulus. Such models provide the basis for drug screening assays to identify molecules that can restore neurite outgrowth within an inhibitory environment.

Another important application for stem cells in vitro is the generation of disease-specific models that can be used to further elucidate the complex molecular mechanisms underpinning specific neurodegenerative disease, such as in Alzheimer's disease and PD, together with potential screening applications for new drug treatments (Figure 3). Disease-specific models can be derived from ES cells through the identification of genetic abnormalities by pre-implantation genetic diagnosis (Mateizel et al., 2006) or pre-implantation genetic screening (Biancotti et al., 2010) during in vitro fertilisation and the subsequent isolation of embryos that would otherwise be discarded. However, as iPSC technology has become more routine, specific disease models are

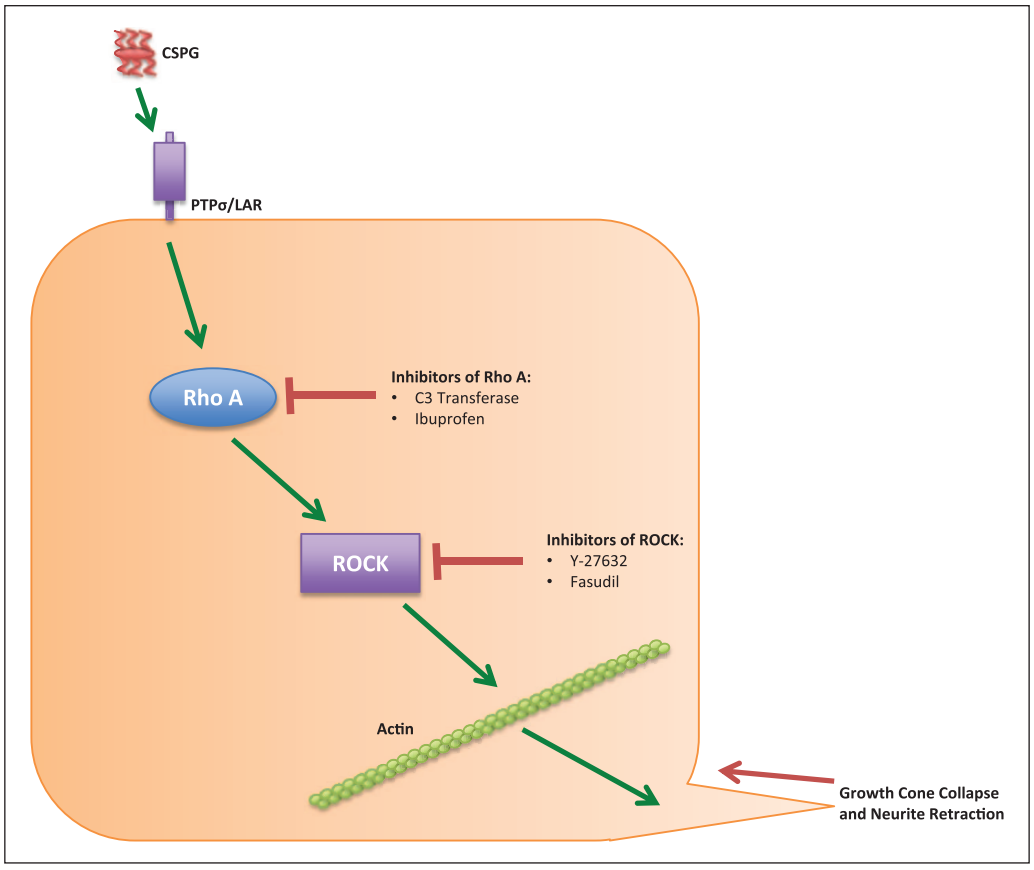

Figure 2. The Rho A signalling cascade is often targeted to induce neurite outgrowth in vitro. In vitro stem cell–based models of neurite outgrowth have provided an opportunity to study the molecular pathways that induce neurite inhibition within a variety of neurological disorders. It is now thought that in the glial scar that forms following damage to the spinal cord chondroitin sulphate proteoglycans (CSPGs) activate Rho A signalling through a receptor-dependent mechanism which ultimately results in the activation of downstream Rho-associated protein kinase (ROCK). Activation of this signalling pathway in turn results in the stabilisation of actin filaments inducing growth cone collapse and neurite retraction. In vitro neurite outgrowth models have allowed for the screening of potential inhibitors of this pathway and have resulted in the identification of compounds that can induce recovery of neurite growth in an inhibitory environment. These include inhibitors of Rho A such as C3 transferase and ibuprofen and also inhibitors of ROCK such as Y-27632 and fasudil.

more commonly derived from somatic cells taken from a patient and reprogrammed into iPSCs for use in in vitro modelling and drug screening (Avior et al., 2016). Fibroblasts taken from patients with neurodegenerative diseases can be reprogrammed using a cocktail of transcription factors to a pluripotent state (iPSC) that can then be differentiated towards a neural lineage to produce cells with a specific disease phenotype. Such cells can subsequently be used in the screening of compounds that can potentially induce neural regeneration and recovery of the disease phenotype (Barmada et al., 2014; Cooper et al., 2012; Hossini et al., 2015; Israel et al., 2012; Liu et al., 2014; Ren et al., 2015; Sareen et al., 2013). This technology also has an application in personalised medicine, as drug efficacy for specific patient conditions can be determined (Avior et al., 2016).

Stem cell–based neuroregenerative therapies

Stem cell differentiation procedures can be used to guide stem cell fate towards a desired lineage, producing a population of specialised cells. This has implications in many degenerative diseases, as a source of cells for transplantation purposes. A major consideration in the use of stem cell derivatives for cell replacement therapies is the purity of the cell population. This is a particularly important consideration as the remaining pluripotent cells in the transplanted population could potentially lead to tumour formation. However, the incidence of tumour formation in the majority of clinical trials appears to be well controlled (Trounson and DeWitt, 2016). Therefore, the use of stem cells in regenerative medicine is becoming a popular area of research with clinical trials underway for the use of this technology in many neurodegenerative diseases including PD and ALS (Trounson and DeWitt, 2016).

ALS is a degenerative disease caused by the death of motor neurons that results in a progressive decline in neuromuscular capacity (Contestabile, 2011). Unlike the genetic form of ALS that has been linked to a mutation in the gene for superoxide dismutase 1 (SOD-1; Kaur et al., 2016), the mechanisms of disease pathogenesis in spontaneous ALS remain poorly understood. However, astrocytes, which in healthy individuals provide metabolic support to the neuron, are thought to adopt a pro-inflammatory phenotype

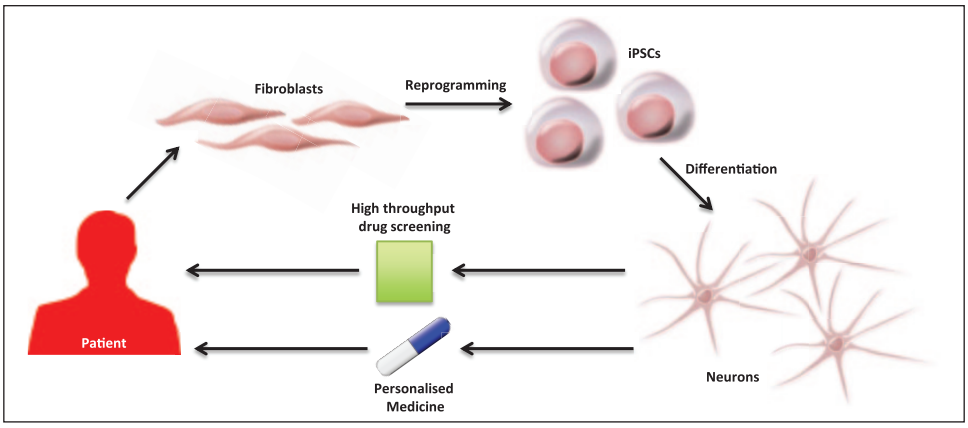

Figure 3. In vitro culture of induced pluripotent stem cells (iPSCs) and their use in drug development. Patient-derived somatic cells such as fibroblasts can be reprogrammed to form iPSCs that in turn can undergo robust differentiation to form mature neurons in culture. This process can be used to produce disease-specific neural subtypes such as neurons from patients with complex neurodegenerative disorders and in vitro models simulating aspects of the disease. These models can then be used for high-throughput screening (HTS) of test compounds that may combat conditions symptomatic of the disorder. iPSC technology also provides advantages in the field of personalised medicine as patient-specific in vitro models of neurological disease can be generated to test the efficacy of specific drug treatments prior to their administration.

in ALS, contributing towards neurotoxicity. For this reason, studies have focused on the generation of astrocytes from pluripotent stem cells and there has recently been approval for a Phase I–II clinical trial based on the transplantation of a glial-restricted progenitor derivative of ES cells (Trounson and DeWitt, 2016). Much of the focus of stem cell therapies for ALS has been to improve the diseased microenvironment and provide neurotrophic support to resident cells (Boulis et al., 2011; Lunn et al., 2009, 2011; Silani et al., 2010). This has been achieved by grafting foetal-derived neuroprogenitor cells that secrete glial-derived neural growth factor into the spinal cord of rats to prevent respiratory failure (Gowing et al., 2014). Another approach that has undergone clinical trials is the intraspinal transplantation of human spinal stem cells that not only secrete growth factors with neuroprotective properties but also differentiate into functional neurons that form synapses with resident neurons (Feldman et al., 2014).

PD is a progressive degenerative disease and movement disorder, clinical symptoms of which may include bradykinesia, rigidity, resting tremor and postural instability, along with cognitive and psychiatric disturbances (Moore et al., 2005). Symptoms result from a selective loss of dopaminergic neurons within the substantia nigra of the brain (Moore et al., 2005). A significant amount of research has focused on the use of stem cell technologies and differentiation procedures to generate and replace lost dopaminergic neurons. However, although A9 dopaminergic neurons are known to be the cell type that is depleted in PD, the exact cell type for transplantation has been under review as patients transplanted with foetal brain tissue have subsequently developed dyskinesia.

Considerable progress has been made over recent years in the generation of functional dopamine neurons from ES cells for transplantation purposes and, when transplanted into rodent models of the disease, they have resulted in restoration of motor function to a level that is similar to foetal tissue transplantation (Ganat et al., 2012; Grealish et al., 2014; Steinbeck and Studer, 2015). In addition to this, transplanted neurons have been found to form synapses with resident neurons, release dopamine and ultimately reduce clinical symptoms associated with the disease (Trounson and DeWitt, 2016). The long-term survival and characterisation of the resultant cell population generated by ES cell differentiation have been important factors to consider in the development of this therapeutic strategy (Ganat et al., 2012; Grealish et al., 2014).

In addition to the generation of dopaminergic neurons from ES cells, parthenogenetic embryonic stem (pES) cell–derived neurons have been approved for Phase I clinical trials for the treatment of PD (Trounson and DeWitt, 2016). Unlike ES cells, pES cells are derived from the chemical stimulation of unfertilised human ova that are unable to develop into normal offspring due to a wide range of developmental and epigenetic defects (Brevini et al., 2012). pES cells offer the ethical advantage over ES cell–based therapies, as viable human embryos are not destroyed in their derivation and they can be used to generate an unlimited supply of neurons for transplantation (Gonzalez et al., 2015). Neurons derived from pES cells transplanted into rodent and non-human primate models of PD have resulted in an increase in dopamine levels without any adverse effects (Gonzalez et al., 2015), suggesting that pES cell–derived neurons may be a suitable candidate for cell replacement therapies in PD.

Huntington's disease (HD) is a progressive neurodegenerative disorder that is untreatable and ultimately fatal. It is an autosomal dominant, inherited disorder that results in the loss of dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP)-32-positive medium spiny projection neurons in the striatum, and therefore the replacement of this cell type with stem cell derivatives is becoming an attractive therapeutic option (Reddington et al., 2014). Foetal striatal tissue transplantation has had mixed results in the treatment of HD, with long-term grafts resulting in a diseased phenotype of the transplanted tissue (Barker et al., 2013; Cicchetti et al., 2009; Steinbeck and Studer, 2015). Research is now focused on the generation of medium spiny neurons from ES cells and iPSCs. Transplantation of such neurons has resulted in limited success, as transplanted cells do not appear to integrate into host neuronal networks (Reddington et al., 2014). It is now thought that transplantation success can be

improved by the inclusion of glial support in the transplanted population (Reddington et al., 2014).

Trauma to both the PNS and the CNS can result in a loss of neural connectivity, either due to the incapacity of the CNS to regenerate, or due to a lesion exceeding the means of regeneration in the PNS. The generation of stem cell–derived neural cells to bridge the injury, replacing lost tissue with the potential to restore function, is becoming an increasingly popular area of research (Trounson and DeWitt, 2016). MSCs due to their ability to differentiate into mature subtypes and secrete factors that form an environment conducive to regeneration have formed the basis of many studies aimed at treating functional deficit in SCI (Varma et al., 2013). The result of MSC treatment in SCI has been mixed, as many studies have used different methodologies of transplantation which has resulted in a varied outcome ranging from improvement in neurological function to no clinical improvement (Varma et al., 2013).

Repair of peripheral nerve injury by many types of stem cell has been evaluated with successful methodologies including the growth of DPSCs in a conduit containing a collagen gel to bridge the gap in a rat facial nerve injury, supporting axonal regeneration (Bhangra et al., 2016). Functional recovery has also been reported in mice with a sciatic nerve defect through the use of a bio-adsorbable conduit containing iPSC-derived neurospheres and a growth factor delivery system (Bhangra et al., 2016). Therapies based on iPSC-derived cellular treatments also have the added benefit of providing patient-specific cell-based therapies, avoiding any immune rejection issues.

Common strategies employed to treat peripheral nerve injury often combine biomaterials with stem cell technology to bridge and populate large nervous lesions. Such materials include graphene, which is both conductive and can enhance neuronal differentiation from populations of NSCs (Li et al., 2013). Solid scaffolds composed of synthetic materials such as polystyrene (Hayman et al., 2004) and polylactide (Melissinaki et al., 2011) have also been described to enhance neural differentiation and promote the growth of neuronal cell types. Integrated approaches to enhance functionality following peripheral nerve injury are essential to ensure that cells are delivered to the site of injury through the most appropriate mechanism.

Conclusion

The ability of stem cells to continually self-renew and produce a defined population of specialised neural cells upon induction of differentiation is an important tool in the treatment of neurodegeneration. Both pluripotent and multipotent stem cell types have important applications in the development of in vitro models of disease pathogenesis, allowing investigation into specific disease mechanisms and the screening and identification of potential new drugs. The generation of neuronal populations from such cells is also an important source of cellular material for transplantation purposes, with early clinical studies showing evidence of beneficial effects in several neurological disorders. This area of stem cell research is particularly active, with promising results in terms of functional recovery following transplantation. Particularly, iPSC technology offers a range of benefits over other types of stem cells, as they can provide an autologous source for patient-specific cell types for transplantation or personalised medicine through drug screening.

The outcome of such work has also demonstrated that much more needs to be considered than just replacing a lost cell type, and that the entire cellular microenvironment and secretory profile of cells should be considered to enable restoration of a healthy nervous system. This is particularly important in terms of complex neurodegenerative diseases such as Alzheimer's disease and PD, as replacing a lost cell type may not permanently restore functionality. For this reason, better understanding of the molecular pathogenesis mechanisms should always be considered simultaneously with cell replacement therapy.

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