Chapter 13
Transplantation of Olfactory Ensheathing Cells in Spinal Cord Injury

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Contents

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Abstract Spinal cord injury is characterized by massive cellular and axonal loss, a neurotoxic environment, inhibitory molecules and physical barriers that hamper nerve regeneration and reconnection leading to chronic paralysis. Transplantation of different types of cells is one of the strategies being examined in order to restore the lost cell populations and to re-establish a permissive environment for nerve regeneration. The mammalian olfactory system is one of the few zones in the body where neurogenesis occurs during the lifetime of the organism, with olfactory neurons being replaced daily with their axons elongating from the peripheral nervous system into the central nervous system to re-establish functional connections. The regenerative ability of this system is largely attributed to the presence of a unique group of cells called olfactory ensheathing cells (OECs). OECs have emerged as an encouraging cell candidate for transplantation therapies to repair the injured spinal cord with multiple animal models showing significant functional improvements and several human trials establishing that the procedure is safe and feasible. Even though the results are promising with some animal models showing remarkable restoration of function, the variability amongst studies in terms of outcome assessments, cell purity, cell culture and transplantation protocols make it difficult to reach firm conclusions about the effectiveness of OEC transplant therapy to treat the injured spinal cord. These variations need to be addressed in order to achieve a more realistic understanding of how the benefits of OEC transplantation enhance the therapeutic outcomes.

Abbreviations

BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CP	Cribriform plate
CSPG	Chondroitin sulfate proteoglycan
DAPI	4’,6-diamidino-2-phenylindole
dBcAMP	Dibutyryl cyclic adenosine monophosphate
FGF	Fibroblast growth factor
GDNF	Glial cell-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
GL	Glomerular layer
HNK-1	Human natural killer-1
IGF	Insulin-like growth factor
LP	Lamina propria
MAG	Myelin associated glycoprotein
NFL	Nerve fibre layer
NGF	Nerve growth factor
Nogo	Neurite outgrowth inhibitory protein
NPY	Neuropeptide Y
NT4	Neurotrophin 4
NT5	Neurotrophin 5

OE	Olfactory epithelium
OEC	Olfactory ensheathing cell
OMgp	Oligodendrocyte-myelin glycoprotein
p75NTR	p75 low-affinity neurotrophin receptor
PNS	Peripheral nervous system
SCI	Spinal cord injury
TROY	TNFRSF expressed on the mouse embryo
VEGF	Vascular endothelial growth factor

13.1 Spinal Cord Injury

Spinal cord injury results in large-scale neuronal loss with very limited capacity for regeneration, leading to chronic paralysis. The main factor hampering recovery is the inability of regenerating spinal cord axons to reach their target (reviewed by Leal-Filho 2011). The pathophysiology of spinal cord injury is divided in two stages: the primary and secondary lesion. The primary lesion is caused by the direct mechanical trauma, i.e. laceration, contusion or compression, resulting in structural disturbances, death of neurons and damage to neural connections. This is followed by ischemia and microvascular damage (Beattie et al. 1997), as well as excessive extracellular glutamate as a consequence of neuronal cell death (Hermann et al. 2001). The high concentration of glutamate and other excitatory amino acids lead to further progressive cell death via excitotoxicity and free radical production (Byrnes et al. 2009). Altogether, the cell death, oxidative stress and inflammatory responses result in massive neuronal and glial cell death (Hulsebosch 2002; Jones et al. 2003).

The secondary lesion is characterized by continued inflammatory immune responses, including cytokine and interleukin secretion by macrophages and neutrophils (Bolton 2005). Furthermore, axon demyelination, as a consequence of oligodendrocyte cell death, results in loss of axonal conduction and subsequently a loss in synaptic communication. Activated astrocytes migrate to the injury site to degrade axonal debris and remove toxic chemicals, but the vast network of activated astrocytes creates a glial scar; a compact structure that becomes a barrier preventing regenerating axons from reaching their target (Bunge et al. 1960; Matthews et al. 1979). Thus, the complex damage resulting from the initial nerve injury leads to an environment that hampers or even completely inhibits neuronal regeneration (Fig. 13.1) (Hulsebosch 2002; Leal-Filho 2011).

Current therapies for spinal cord injury do not lead to significant neural regeneration and functional recovery. Most of these therapies have aimed to minimize the post-traumatic cell damage but fail to achieve the re-establishment of neuronal connections. Drug therapy is generally applied immediately following trauma to treat inflammation and initial degeneration (reviewed by Stahel et al. 2012; Batzofin et al. 2013; Hurlbert et al. 2013). This treatment is often followed by long term therapies aimed at promoting axonal growth and neutralising the toxic environment at the injury site. A major factor hampering axonal regeneration

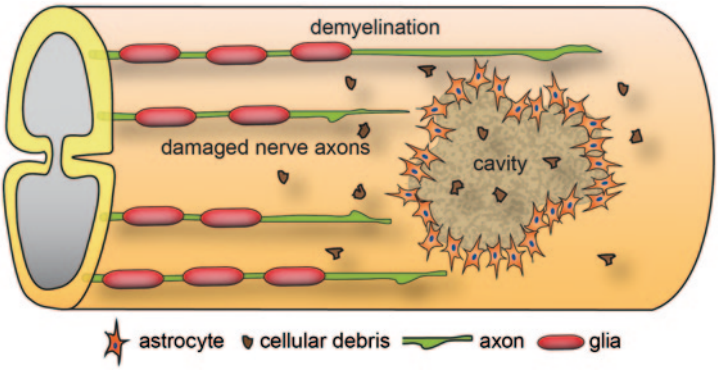

Fig. 13.1 Detrimental effects of secondary spinal cord injury events at the cellular and molecular level. Neurons are shown in *green*; glia in *red*. Injury to the spinal cord leads to (1) apoptosis and cell death (neurons and glial cells) with accumulation of cell debris and the generation of a toxic environment due to high extracellular glutamate concentrations and the presence of free radicals; (2) formation of a glial scar which creates a physical barrier consisting of activated astrocytes. The resultant effect is that damaged axons are unable to regenerate and communicate due to presence of inhibitory molecules, loss of myelin and the physical barrier

following spinal cord injury is the down-regulation of endogenous neurotrophins and one method that has shown promise is the injection of neurotrophins at the site of injury to replace the lost endogenous neurotrophins (Hulsebosch 2002). Peripheral glial cells can produce many growth factors and hence transplanting glia to the injury site is an even more promising approach as these cells can integrate with endogenous cells and scar tissue, producing a more long-term growth-promoting environment (Yan et al. 2001; Feron et al. 2005; Cao et al. 2007; Centenaro et al. 2011). Similarly, pluripotent stem cells can be transplanted to the injury site, potentially resulting in neuronal regeneration and production of glial cells. This method is still experimental, but has resulted in promising functional outcomes in animals (reviewed by Antonic et al. 2013). Further, manipulation of gene expression to block production of growth-inhibitory and toxic molecules has also resulted in some promising functional outcomes (reviewed by Leal-Filho 2011). Overall, however, while these therapeutic interventions have led to some positive outcomes, to date, none have produced a significant functional recovery in humans (Lim and Tow 2007; Leal-Filho 2011).

13.2 Endogenous Glial Cells and Their Role in Spinal Cord Injury

Glial cells are the most abundant cells in the nervous system. They are closely associated with neurons and were previously described simply as supportive nervous tissue. A deeper understanding of glial cell biology, however, has demonstrated that

glial cells exhibit a multitude of complex roles and are essential for the development and function of the entire nervous system (Jessen 2006). Glial cells are a heterogeneous population of cells that differ in developmental origin, molecular composition, structure and specific behaviour, and exist together with neurons and other cells in an integrated and co-dependent system (Chung and Barres 2012). Throughout the nervous system, glial cells have crucial roles in axonal extension and guidance, protection against mechanical, chemical and oxidative injury, as well as preservation of the electrical and chemical balance of all neurons (Ndubaku and de Bellard 2008).

Glial cells can be broadly classified as being either central nervous system glia or peripheral nervous system glia. In the mature central nervous system (CNS), there are two major types of glial cells of neural origin; astrocytes and oligodendrocytes. Other types of CNS glial cells exist that originate from non-neuronal precursors; microglia constitute part of the innate immune system and originate from macrophage lineages (Chugani et al. 1991). In the peripheral nervous system (PNS), Schwann cells constitute the main glial cell type, with the exception of the olfactory nervous system, which is populated by specialized glia termed olfactory ensheathing cells (OECs).

Astrocytes play a critical role in the function and homeostasis of the CNS. They are required for the formation and maintenance of the blood-brain barrier, provide support for axonal extension and play an active role in neuronal signalling by exchange of ions and production of neurotransmitters, as well as cell adhesion and synapse signalling molecules (Kriegstein and Gotz 2003). Astrocyte–neuron interactions are known to secure the survival and normal function of neurons (Jessen 2004). Numerous studies have demonstrated that astrocytes play important neuroprotective roles, in neurodegenerative disorders (reviewed by Singh et al. 2011; Cabezas et al. 2012) and they have the ability to promote neuronal survival by protecting against reactive oxygen species and other stressors (Lopez et al. 2007).

After spinal cord injury, astrocytes respond rapidly by migrating to the injury site, where they proliferate and form a compact structure, a glial scar, to preserve the blood-brain barrier, protecting the CNS and maintaining the adequate ionic environment necessary for nerve function. However, the glial scar eventually becomes a physical barrier that stops damaged axons from regenerating and reconnecting (Fig. 13.1) (Leal-Filho 2011). Furthermore, astrocytes respond to neuronal injury by increasing their proliferation and by secreting glycoproteins such as chondroitin sulfate proteoglycans (CSPG), which act to inhibit axon elongation (Table 13.1) (Qiu et al. 2002; Su et al. 2009).

Oligodendrocytes are morphologically similar to astrocytes, albeit with fewer and smaller branched processes. They play different roles in the modulation of neuronal function as well as the regulation of proliferation, survival and differentiation of neurons (Jauregui-Huerta et al. 2010) The most important role of oligodendrocytes, however, is to myelinate axons. The myelin sheet provides electrical insulation around the nerve fibres, speeding the transmission of electrical signals (Jessen 2004). The myelin layer also protects the axons by creating a “safe chamber”, resembling a growth-promoting channel through which

Table 13.1 Glial cell response to spinal cord injury

Type of Glia	Response in spinal cord injury event	Reference
Astrocytes	Removal of toxic chemicals (glutamate). Proliferation and secretion of neuroprotective but growth-inhibitory factors. Formation of glial scar	(Qiu et al. 2002; Su et al. 2009)
Oligodendrocytes	Massive death due to high glutamate concentrations. Production of glycoproteins with Nogo-receptor affinity that will suppress myelin production	(Jones et al. 2003; Jessen 2004; Arevalo et al. 2010)
Microglia	Initially phagocytosing debris and producing neuroprotective factors. Over time become neurotoxic and growth-inhibitory due to constant activation	(Chatzipanteli et al. 2002; Pearse et al. 2003; Block and Hong 2005; Kigerl et al. 2009)
Schwann cells	Cells de-differentiate to an immature state, lose their myelin sheath conformation and migrate from the periphery into the injury site in the CNS, where they participate in endogenous repair processes by expression of neurotrophic factors	(Farbman and Squinto 1985; Jessen 2004; Oudega and Xu 2006)

the axon extends. After spinal cord injury, populations of oligodendrocytes are rapidly affected by high levels of glutamate and massive cell death follows. Oligodendrocytes that do survive produce neurite outgrowth inhibitor (Nogo), myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp) (Table 13.1), proteins that bind to the Nogo receptor, repressing myelin production and affecting axonal outgrowth and neuronal synapses (Jones et al. 2003). Microglia, which are essentially macrophages present within the CNS, respond to injury by migrating to the injury site, where they phagocytose debris, secrete a range of both pro- and anti-inflammatory cytokines and growth factors which initially have a neuroprotective effect. Over time, however, microglia near and in the injury site respond to the constant prolonged activation by secreting molecules that are growth-inhibitory or toxic, thus repressing axonal regeneration (Chatzipanteli et al. 2002; Pearse et al. 2003; Block and Hong 2005). The majority of the activated microglia transition to the M1 type, which can directly induce neuronal death (Kigerl et al. 2009; Gao et al. 2013). Thus, together with the glial scar, the local environment at a CNS injury site inhibits long-term neuronal extension and regeneration.

The PNS differs dramatically from the CNS in terms of capability to regenerate itself after injury. In contrast to central nerves, peripheral neurons in general regenerate after injury, unless large nerves have been completely severed. Schwann cells play an active role in repair of peripheral damaged nerves as a consequence of their ability to differentiate, migrate, proliferate, secrete growth factors, and produce myelin. Schwann cells are classified as either myelinating or non-myelinating. Myelinating Schwann cells enwrap individual peripheral axons, forming the myelin sheath, whereas the non-myelinating type have metabolic and mechanical support

functions (Jessen 2004). After spinal cord injury, Schwann cells migrate from the periphery into the injury site within in the CNS, and participate in endogenous repair processes (Table 13.1). They re-enter the cell cycle, lose their myelinating phenotype and de-differentiate into an immature state, and begin to express trophic factors and cell adhesion molecules that provide a more favourable environment for axon regeneration and extension (Oudega and Xu 2006).

One approach to improve the outcomes after spinal cord injury is to transplant glial cells into the injury site to reduce inflammation, and which will help form a glial bridge across the injury site and thereby promote axon extension. The glial of the PNS system, Schwann cells and OECs, have been trialled in animal models and in humans with various outcomes. The OECs have unique characteristics that may confer an advantage over other glial cell types for transplant therapies.

13.3 The Mammalian Olfactory Nervous System

The mammalian olfactory nervous system is one of the few regions in the CNS in which neurogenesis continuously occurs during the lifetime of the organism (Mackay-Sim and Kittel 1991a, b). The primary sensory neurons of the olfactory system line the dorsal/caudal nasal epithelium and are directly exposed to the environment (Fig. 13.2). The neurons are subjected to attack and destruction by bacterial (St John et al. 2014) and viral pathogens as well as toxins within the air and thus need to be replaced throughout life. Whilst the average life-span of olfactory neurons has not been clearly determined in humans, mouse olfactory neurons generally live for one to three months. Neurons that degenerate are rapidly replaced by new neurons arising from progenitor cells that line in the basal layer of the olfactory mucosa (Mackay-Sim and Kittel 1991b), a process that occurs throughout life (Ramon-Cueto and Santos-Benito 2001).

The primary olfactory system comprises the olfactory mucosa and the bundles of olfactory nerves that project into the olfactory bulb. Stem cells that line basal layer of the olfactory epithelium give rise to the primary olfactory sensory neurons which migrate apically to populate the olfactory epithelium (Fig. 13.2). Olfactory sensory neurons have a bipolar morphology with a single dendrite extending onto the surface of the epithelium and a single axon projecting to and terminating in the olfactory bulb. Each olfactory neuron expresses a single odorant receptor type with the neurons mosaically distributed throughout the epithelium, but the axons of the same odorant receptor type converge to the same targets within the olfactory bulb (Vassar et al. 1994; Mombaerts et al. 1996). To reach their targets in the olfactory bulb, the axons of the olfactory sensory neurons project through the lamina propria that underlies the olfactory epithelium and pass through the bony cribriform plate to enter the nerve fibre layer which is the outer layer of the olfactory bulb and within the CNS. Thus, new axons must constantly traverse the PNS-CNS border and find their correct targets inside the olfactory bulb (Valverde et al. 1992; Tennent and Chuah 1996; Chehrehasa et al. 2010). The constant ability of olfactory

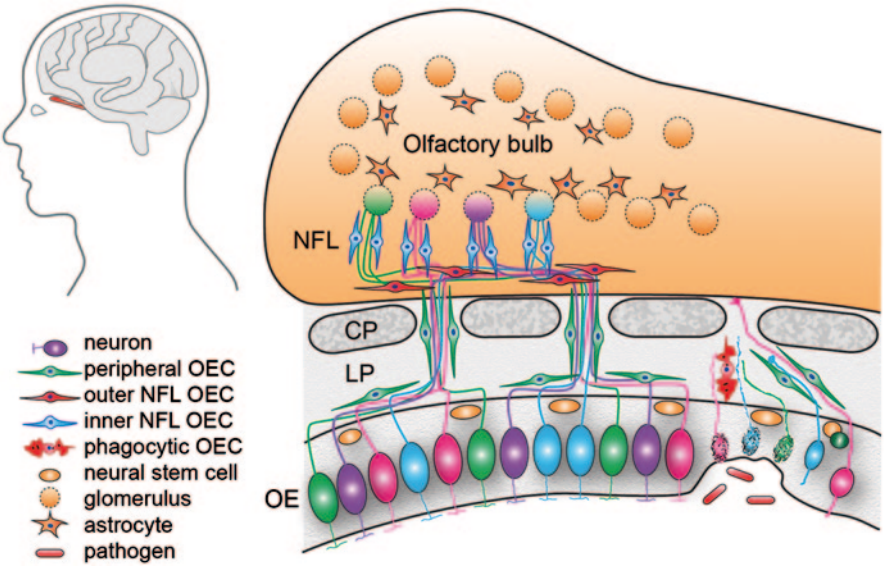

Fig. 13.2 Anatomical organisation of the olfactory system. Primary olfactory sensory neurons lie within the olfactory epithelium (*OE*). Their axons project through the cribriform plate (*CP*) and enter the olfactory bulb where they terminate in their target glomeruli. *OEC*s within the lamina propria (*LP*) encase the bundles of numerous different axons as they project to the olfactory bulb. In the outer nerve fibre layer (*NFL*) of the olfactory bulb, the OECs (*red*) aid the defasciculation and sorting of the different axons. In the inner layer of the nerve fibre layer the *OEC*s (*blue*) assist with the refasciculation and targeting of similar axons to their targets. Astrocytes form a barrier around the glomeruli (*dashed circles*). The olfactory sensory neurons within the *OE* are subjected to toxic molecules within the inhaled air and pathogens such as bacteria and viruses which can result in the death of the neurons (spotted neurons). *OEC*s phagocytose the debris from the degenerated axons. Stem cells lining the basal layer of the *OE* replenish the neuron population which project axons through channels maintained by the *OEC*s

neurons to regenerate and the unique ability of olfactory axons to extend across the PNS-CNS boundary are attributed to the presence of the glia of the olfactory system, called OECs.

13.3.1 Olfactory Ensheathing Cells—the Glia of the Olfactory System

OECs arise from neural crest (Barraud et al. 2010) and they are constantly in close contact with the axons of olfactory neurons all the way from the nasal epithelium to the outer layer of the olfactory bulb. OECs ensheathe the axons of olfactory neurons by the extension of cytoplasmic processes (Chuah and Zheng 1992; Tennent and Chuah 1996) followed by the fasciculation of the axons into larger bundles which ultimately join to form the olfactory nerve (Whitesides and LaMantia 1996).

In contrast to Schwann cells, which in the process of myelination enwrap one single axon (Fig. 13.3), OECs ensheathe bundles of multiple axons by projecting extensive thin cytoplasmic processes around and between the numerous axons within the fascicles (Fig. 13.2; 13.3).

OECs also have a role in promoting axon growth and are known to secrete numerous axon growth promoting factors, such as nerve growth factor, brain derived neurotrophic factor and neuregulins (Boruch et al. 2001). During development, OECs proliferate and migrate ahead of axons or surround the growth cones of axons (Tennent and Chuah 1996; Chehrehasa et al. 2010). Loss of OECs from the olfactory nerve during development results in poor axon growth and targeting (Barraud et al. 2013) which demonstrates that OECs are crucial to the growth and maintenance of axons.

OECs are also thought to be crucial for regeneration during normal turnover of olfactory sensory neurons or after large-scale infection by bacteria and viruses, or major injury. Bacterial infection can lead to the death of olfactory sensory neurons and subsequently their axons (Fig. 13.2; St John et al. 2014), or injury can directly lead to the destruction of the axons (Graziadei et al. 1978; Chehrehasa et al. 2010). The debris from the degenerated axons must be removed but unlike other areas of the body where cells of the immune system usually clear away debris, in the olfactory system this function primarily relies on the OECs (Su et al. 2013). OECs have been shown to continuously phagocytose debris arising from the degenerating axons that occurs during normal turnover of neurons or after widespread injury (Wewetzer et al. 2005; Su et al. 2013). OECs are also able to phagocytose bacteria and thereby protect the olfactory pathway from infection (Wewetzer et al. 2005; Leung et al. 2008; Panni et al. 2013).

OECs form a three-dimensional structure resembling a tunnel through which the axons extend (Fig. 13.3; Li et al. 2005). These structures remain intact even after olfactory axons have degenerated completely following large-scale injury to the olfactory epithelium (Li et al. 2005). However, after large scale injury, OECs can proliferate not only locally around the injury but also from precursors that are present in the olfactory mucosa after which they then migrate along the olfactory nerve (Chehrehasa et al. 2012). By maintaining open channels through which regenerating axons can extend and by responding to injury by proliferating and migrating to the region of need, the OECs with their axon growth-promoting properties provide the structure and support needed for the continuous successful regeneration of the olfactory system.

OECs produce numerous growth factors such as fibroblast growth factor (FGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), as well as neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), neurotrophin 4 (NT4) and NT5; as well as extracellular matrix and cell adhesion molecules including laminin, collagen, galectin-1, heparin sulfate proteoglycans, glial-derived nexin and N-cadherin (Doucette 1990; Doucette and Devon 1993; Chuah and Teague 1999; Kafitz and Greer 1999; Tisay and Key 1999; Boruch et al. 2001; Woodhall et al. 2001; Woodhall et al. 2003; Chuah

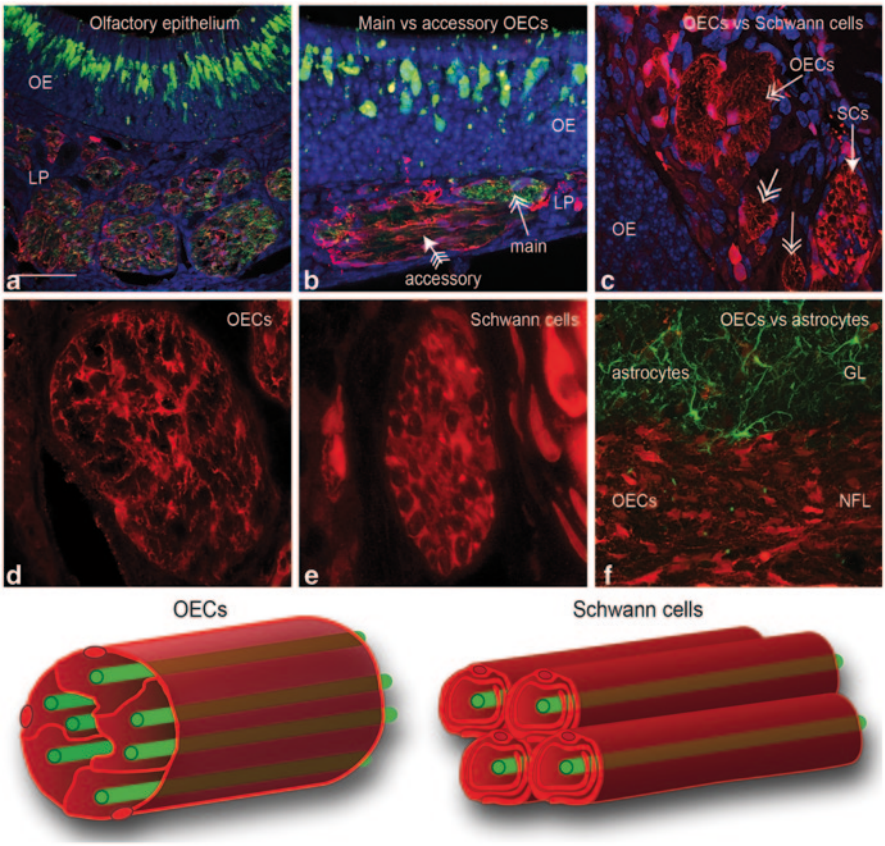

Fig. 13.3 The different populations of glia in the olfactory system. Panels a-f show coronal sections through the olfactory system of a transgenic reporter mouse (OMP-ZsGreen X S100ß-DsRed; Windus et al. 2007; Ekberg et al. 2011) that expresses ZsGreen fluorescent protein in olfactory neurons and DsRed fluorescent protein in glia. **a** The primary olfactory neurons (*green*) within the olfactory epithelium (*OE*) project axons into the lamina propria (*LP*) where they coalesce into fascicles wrapped up by *OEC*s (*red*). Nuclei (*blue*) are stained with DAPI. **b** Along parts of the septum in the nasal cavity, the axon fascicles of the main olfactory system are adjacent to axon fascicles of the accessory (pheromone) olfactory system. **c** Branches of the trigeminal nerve also innervate the nasal cavity; the trigeminal nerve axons are encased by Schwann cells with the trigeminal nerves running adjacent to the main olfactory nerve fascicles that are encased by *OEC*s (*double-headed arrows*). **d** The cell bodies of the olfactory glia are mainly restricted to the periphery of the axon fascicles with their processes permeating the central regions of the axon fascicle. **e** Schwann cells of the trigeminal nerve form tube-like encasing of individual axons. **f** In the olfactory bulb, *OEC*s (*red*) in the nerve fibre layer (*NFL*) form a barrier with the astrocytes (*green*; GFAP immunostaining) in the glomerular layer (*GL*). **g** Schematic of the ensheathment of olfactory axons by *OEC*s. The cell bodies of *OEC*s are largely restricted to the exterior and the processes of the *OEC*s penetrate the internal areas of the nerve bundle where they surround numerous olfactory axons. **h** Schematic of Schwann cell ensheathment of other peripheral nerves in which individual axons are myelinated and encased by Schwann cells. Scale bar is 65 µm in a; 50 µm in b; 30 µm in c; 20 µm in d; 15 µm in e; 40 µm in f

et al. 2004; Chung et al. 2004; Vincent et al. 2005b; Mackay-Sim and St John 2011). These OEC-derived factors are likely to play an important role in nerve repair and regeneration processes as well as neutralization of toxic cell environments due to the excess of free radicals and neurotransmitters such as glutamate (Doucette 1995; Ramon-Cueto 2000; Woodhall et al. 2001; Woodhall et al. 2003; Ramon-Cueto 2011).

13.3.2 Differences Between Olfactory Ensheathing Cells and Schwann Cells

Originally, OECs were referred to as Schwann cells of the olfactory system (Doucette 1984), but their distinctive characteristics separated them from other glial cell types to such an extent that they were classified as an individual glial type. OECs possess features of both CNS and PNS glia in terms of morphology and molecular profile, consistent with their location to both the central and peripheral part of the olfactory nervous system and their ability to cross the PNS-CNS interface. Developmentally, OECs and Schwann cells are of neural crest origin (Barraud et al. 2010), in contrast to astrocytes, which arise from radial glia of neuroepithelial origin (Kriegstein and Gotz 2003). OECs are known to express a number of different proteins found in either Schwann cells or astrocytes. For example non-myelinating Schwann cells and OECs (except those in the inner nerve fibre layer of the olfactory bulb) present immunoreactivity for the p75 low-affinity neurotrophin receptor ($p75^{NTR}$) (Ramon-Cueto 2000).

Whilst similarities between Schwann cells and OECs are evident, one particularly important difference exists in the ability to interact with astrocytes. In contrast to Schwann cells, OECs interact freely with astrocytes, without causing detrimental effect on the astrocyte population (Lakatos et al. 2000). This specific feature is of great interest for nerve regeneration therapies where both populations (OECs and astrocytes) interact at an injury site (Chuah et al. 2011). When OECs are confronted with astrocytes in spinal cord injury sites, astrocyte processes, which form the glial scar, alter their morphology to create a bridging pathway with OECs that allow severed axons to extend across the lesion establishing functional connections (Ramer et al. 2004; Li et al. 2012).

In contrast to Schwann cells, OECs migrate ahead of the regenerating axons, extending their processes to provide a cellular pathway that facilitate axonal extension and adhesion (Tennent and Chuah 1996; Chehrehasa et al. 2010). OECs increase their migration ability by the formation of bigger and thicker processes (Valverde et al. 1992), maintaining a continuous ensheathment of the axons during the regeneration process and leading to enhanced axon growth (Chehrehasa et al. 2010). The capacity of OECs to promote olfactory system renewal and regeneration, as well as their capacity to bridge, enter, and interact with cells of injured host tissue, constitute key factors contributing to the increasing interest in the use of transplanted OECs as therapeutic candidates in spinal cord injury treatments.

13.4 Use of Glial Cells in the Treatment of Spinal Cord Injuries

Re-establishment of nerve connections after spinal cord injury depends of the ability of axons to extend along a pathway to reach their targets. This living pathway consists of glial cells, which provide a dynamic channel through which axons can extend towards their targets (Ramer et al. 2004; Li et al. 2012). After spinal cord injury, the severed nerves are able to survive and sprout locally. However, they are unable to elongate and re-establish the connections, primarily because the glial pathway is altered, blocked and sometimes completely lost. Consequently, a primary objective in the treatment of spinal cord injury is re-establishment of the glial pathway. Transplantation of glial cells into the injury site is therefore a promising therapeutic approach for repair spinal cord injury (Oudega and Xu 2006).

Glial cell transplantation addresses many of the challenges that must be overcome for successful functional improvement, including (1) re-establishment of a growth-promoting environment, (2) replacement of lost cell populations (neurons and glia), and (3) facilitation and promotion of axonal regeneration and extension. Pioneering studies have established that transplantation of glial cells can improve axonal repair, enhance re-growth of damaged nerve cells and improve functional recovery (Yan et al. 2001; Santos-Benito and Ramon-Cueto 2003). Additionally, glial cells have the potential to produce neurotrophic molecules that activate axon regeneration and extension (Jones et al. 2003; Feron et al. 2005).

Different types of glial cells have been investigated as treatment for spinal cord injury including Schwann cells from peripheral nerves and OECs. Schwann cells have been trialled for transplantation due to the important role they play in axon regeneration and myelination. Schwann cells transplanted to the damaged spinal cord can stimulate regeneration of damaged neurons, presumably due to the production of neurotrophic factors (Park et al. 2010), and can also enhance axon remyelination and extension (Lavdas et al. 2010; Flora et al. 2013). However, the axonal regeneration has thus far been limited to restricted areas because Schwann cells have failed to migrate considerable distances into the injured tissue (Lankford et al. 2008). The limited migration is most likely due to unfavorable interaction between the transplanted Schwann cells and host astrocytes (Li et al. 2012). Additionally, Schwann cells have been reported to inhibit myelination by the secretion of connective tissue growth factor, whereas OECs do not (Lamond and Barnett 2013). Thus, Schwann cells may not be the optimal cell type for transplantation therapies due to their poor migration properties, inability to freely interact with endogenous glia and expression of inhibitory molecules.

13.4.1 Transplanted OECs in Spinal Injury Models

Implantation of OECs to promote repair after spinal cord injury have been performed in a variety of spinal cord injury animal models (Table 13.2). Most of these

Table 13.2 Recent examples of in vivo transplantation of olfactory ensheathing cells

First Author (year)	Species	Transplanted cells/tissue	Purity of OECs	Main Outcomes
Richter (2005)	Mouse	Lamina propria Olfactory Bulb	92% p75 positive cells	Stimulation of outgrowth of axon sprouting. Enhanced angiogenesis LP derived OECs superior ability to migrate
Collazos-Castro (2005)	Rats	Olfactory Bulb derived OECs	90% p75 positive cells	Partial improvement of motor function. No improvement in axonal regeneration
Feron (2005) Mackay-Sim (2008)	Human	Lamina propria derived OECs	76–88% p75 positive cells 95% S100 and GFAP positive cells	Transplantation is feasible and is safe up to 3 years of post-implantation. No deterioration in neurological or functional level
Lu (2006)	Rats	Lamina propria derived OECs	97% p75 positive cells	Partial Improvement on axonal regeneration. No difference compared with fibroblast
Huang (2006)	Human embryos	Olfactory bulb derived cells	n/a	Neurological functional improvement after transplantation
Lima (2006)	Humans	Whole layer olfactory mucosa	n/a	Transplantation is feasible, relatively safe, and potentially beneficial. Recovery of bladder sensation and improvement in motor function scores
Toft (2007)	Rats	Olfactory Bulb	98% p75 Positive cells	Improvement on spinal cord function in sensory pathways
Yamamoto (2009)	Rats	Olfactory mucosa	Mixed culture (5% p75 positive)	Restored directed fore-paw retrieval but not axon regeneration observed
Munoz-Quiles (2009)	Rats	Olfactory Bulb	n/a	Progressive improvement in motor function and axonal regeneration
Salehi (2009)	Rats	Embryonic stem cells + Olfactory Bulb OECs	95% p75 positive cells	Neural regeneration, neuron survival and partial functional recovery
Chhabra (2009)	Humans	Whole layer olfactory mucosa	n/a	Procedure is relatively safe and feasible. No efficacy could be demonstrated

Table 13.2 (continued)

First Author (year)	Species	Transplanted cells/tissue	Purity of OECs	Main Outcomes
Aoki (2010)	Rats	Whole layer olfactory mucosa	n/a	Partial improvement in motor function and axonal regeneration
Ma (2010)	Rats	neurotrophin-3 genetically modified Olfactory bulb derived OECs	95% p75 and S100ß positive cells	Effective improvement of axonal regeneration and motor function OECs able to produce NT-3 in vivo
Amemori (2010)	Rats	Lamina propria derived OECs + cAMP infusion	75% p75 positive cells	Improvement motor function Axon regeneration, sprouting and branching Reduce astrocytic hypertrophy
Ziegler (2011)	Rats	Olfactory bulb	n/a p75 Immuno purification	Improvement in hind limb function, injection of OEG facilitated regeneration of axons across a complete mid-thoracic spinal cord transection.
Centenaro (2011)	Rats	Lamina propria derived OECs	n/a	Discrete motor improvement, improved tissue sparing and sprouting
Zhang (2011)	Rats	Lamina propria derived OECs	n/a	Disappearance of the lesion cavity and integration of repaired tissue Activation of host Schwann cells, improved myelination
Novikova (2011)	Rats	Olfactory bulb derived OECs	93–95%	Improvement in motor function and neuronal regeneration Aged cell are less effective
Tharion (2011)	Rats	Olfactory lamina propria derived OECS + Olfactory Nerve Fibroblasts (ONL)	Around 50% p75 positive cells +50% fibronectin positive cells (ONL)	Improvement in diaphragm activities Motor function and axonal regeneration improved Disappearance of the lesion cavity

Table 13.2 (continued)

First Author (year)	Species	Transplanted cells/tissue	Purity of OECs	Main Outcomes
Stamegna (2011)	Rats	Lamina propria derived OECs	90% p75 positive cells	Improvement in axonal regeneration and motor function. Improvement in diaphragm and phrenic nerve activities
Granger (2012)	Dogs	Olfactory mucosa	50% p75 positive cells	Significantly better fore–hind coordination. Effects are likely to be on local intraspinal circuitry
Huang (2012)	Human embryos	Olfactory bulb derived cells	n/a	Treatment feasible after more than 3 years. Improvement of neurological function. No adverse effects noted
Tabakow (2013)	Humans	Olfactory mucosa	20–50% S100 positive cells	Improvement of neurological function observed. No adverse effects noted
Mayeur (2013)	Rats	Lamina propria and olfactory bulb	>97% p75	Improved axon regrowth and reduced scar formation
Toft (2013)	Rats	Olfactory bulb	>97%	Reduced astrocytic hypertrophy
Torres-Espin (2014)	Rats	Olfactory bulb derived cells	75% p75/S100 positive cells	Protection/preservation of tissue around injury site

studies have shown that in rodents with spinal cord injury, OEC transplantation promotes the regeneration of axons (Bartolomei and Greer 2000; Gudino-Cabrera et al. 2000; Ramer et al. 2004) and improves functional restoration of breathing and climbing ability (Li et al. 2003; Su and He 2010; Stamegna et al. 2011). In a study of spinal-injured dogs, the transplantation of OECs and fibroblasts restored significant movement in some dogs probably through the restoration of local circuitry, which clearly demonstrates that the procedure has high potential (Granger et al. 2012). Some studies, however, concluded that transplantation of OECs into injured spinal tract did not lead to any detectable difference in axonal extension and functional outcomes (Collazos-Castro et al. 2005; Lu et al. 2006; Chhabra et al. 2009). Discrepancies between results may be attributed to different variables such as the exact nature of spinal cord injury, OEC isolation and culture protocols, transplantation procedure (site of the OECS transplantation and the time after injury that the transplantation is performed) and methodology used to assess outcome.

Numerous studies have reported significant functional improvements following transplantation of OECs. In rodents, OEC transplantation improved or preserved local circuitry as detected electrophysiologically (Toft et al. 2007), and has improved fore-paw movement (Yamamoto et al. 2009), motor function (Munoz-Quiles et al. 2009; Centenaro et al. 2011; Stamegna et al. 2011), hindlimb function (Salehi et al. 2009; Amemori et al. 2010; Aoki et al. 2010; Ziegler et al. 2011), plantar function (Amemori et al. 2010), locomotor function (Ma et al. 2010) and diaphragm activity (Tharion et al. 2011). Thus depending on the type of injury and the assessment of particular functional tests, OEC transplantation can improve functional outcomes.

Corresponding anatomical outcomes after OEC transplantation have also been detected with the majority of studies focusing on axon growth and reduction in cavity formation and astrocytic scarring (Amemori et al. 2010; Centenaro et al. 2011; Tharion et al. 2011; Zhang et al. 2011; Toft et al. 2013; Torres-Espin et al. 2014). The reduction in cavity formation and astrocytic scarring are considered important features as they preserve the axonal circuitry and reduce the formation of physical barriers that prevent axonal growth. In these situations, OECs could be considered to indirectly promoting axon growth as they are enhancing the local environment to make it subsequently amenable to axon growth across the injury site.

However, anatomical improvements are not always detected despite functional recovery being observed. Significant restoration of fore-paw retrieval but not axon regeneration (Yamamoto et al. 2009) may indicate local circuitry changes outside of the immediate injury site as has been suggested for restoration of function in dogs (Granger et al. 2012) or may indicate improved preservation of function of the spared axons.

The timing of transplantation of OECs may also be important for anatomical and functional outcomes. Acute transplantation of OECs may be preferable as the potential ability of OECs to reduce cavity formation and reduce the creation of an inhibitory environment would likely promote axon regeneration. Indeed, acute transplantation of OECs has shown higher functional and anatomical outcomes compared to delayed transplant (Lopez-Vales et al. 2006). However, other studies have not detected significant differences in outcomes for acute versus delayed trans-

plantation of OECs (Munoz-Quiles et al. 2009; Centenaro et al. 2011; Torres-Espin et al. 2014). Despite this, it would seem reasonable that differences in efficacy and outcomes of acute versus delayed treatment may be revealed if improvements in the experimental conditions were made and the purity of the cell preparations and co-transplanted cells was optimized (as discussed below).

What are the mechanisms by which OECs promote regeneration of the spinal cord? OECs transplanted into spinal cord lesions promote regeneration of axons across the lesion, remyelination of axons (although the mechanism remains unknown) and resumption of numerous cellular functions (Ramon-Cueto 2000; Santos-Benito and Ramon-Cueto 2003; Ramer et al. 2004; Li et al. 2012). In particular, OECs provide a physical "bridge" which assists directional regeneration of severed axons across injury sites (Ramon-Cueto and Nieto-Sampedro 1994; Boruch et al. 2001; Resnick et al. 2003) which is consistent with the role of OECs within the injured olfactory system where it has been shown that OECs migrate ahead of axons and that superior axon regeneration occurs when a substantial OEC environment has been created (Chehrehasa et al. 2010). Different factors such as GDNF and NGF have been identified to regulate migration of OECs (Cao et al. 2004; Windus et al. 2007). For example, when OECS are genetically modified to express higher levels of GDNF they display a superior ability to promote axonal growth (Cao et al. 2004). On a cellular level, GDNF acts by stimulating the behaviour of the peripheral lamellipodial waves, increasing cell-cell contact and contact-dependent migration (Windus et al. 2007). Indeed, it has been demonstrated that the motility of OECs in vitro is directly responsible for the apparent motility of axons due to the preference for axons to adhere to OECs (Windus et al. 2011). Thus the migration of OECs across the spinal cord injury site creates a physical glial bridge rich in axon growth promoting factors which together is likely to enhance axon regeneration.

OECs are also thought to be superior to other glial cell types in their interactions with astrocytes. OECs do not tend to form barriers with astrocytes but instead intermix with the astrocytes and thereby reduce the formation of the astrocytic scar (Ramon-Cueto and Avila 1998; Lakatos et al. 2000; Fairless and Barnett 2005; Santos-Silva et al. 2007). Again, this is consistent with their in vivo role in the olfactory system where OECs of the inner nerve fibre layer interact extensively with the astrocytes that populate the more internal layers of the olfactory bulb (Fig. 13.3f; Lakatos et al. 2000).

The phagocytic activity of OECs (Wewetzer et al. 2005; Su et al. 2013) may also improve the local environment of the injury site by removing cellular debris and thereby reducing the inhibitory factors that prevent axon regeneration. It has been clearly shown that OECs phagocytose axon debris within the olfactory system and in vitro (Wewetzer et al. 2005; Su et al. 2013), as well as within the injured spinal cord (Lankford et al. 2008).

In summary, the likely benefits of transplantation of OECs are that they migrate across the injury site to form a glial bridge rich in axon growth promoting factors, they reduce cavity formation, they interact with astrocytes to reduce scar formation, and they likely phagocytose axon debris from the damaged cells to rapidly create an environment favourable for axon regeneration (Fig. 13.4).

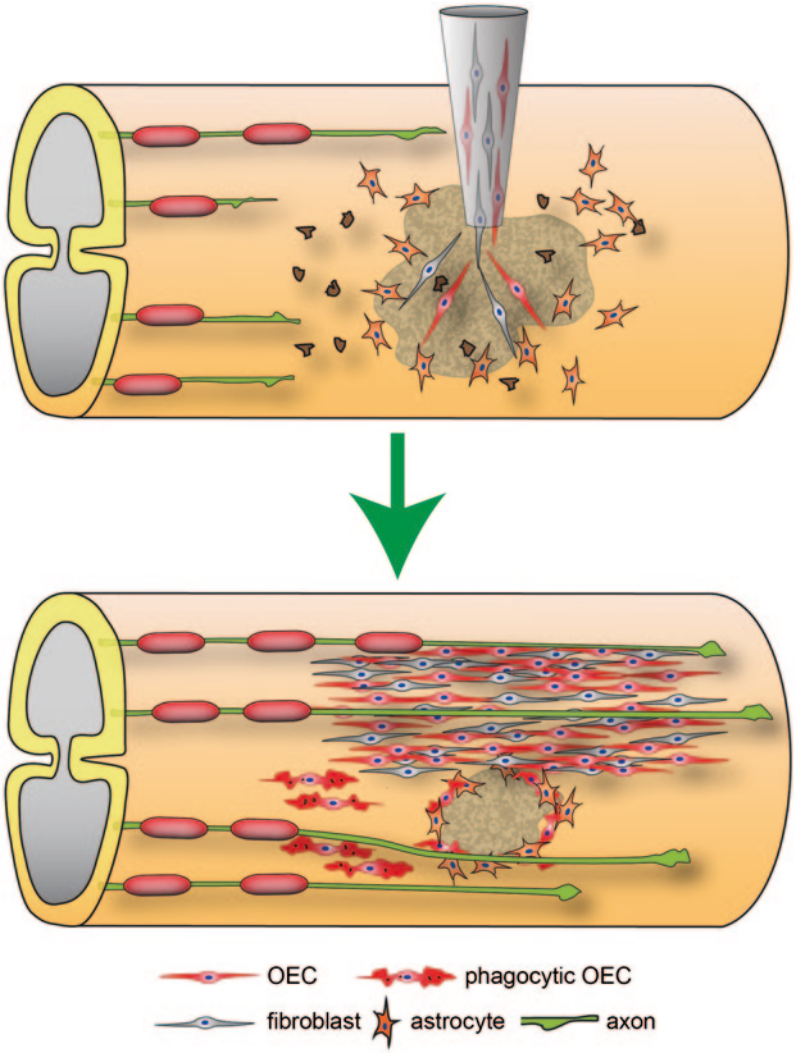

Fig. 13.4 The potential therapeutic use of OECs for spinal cord repair. OECs (*red*) can be transplanted with fibroblasts (*grey*) into the injury site. Strategies that enhance the integration, proliferation and migration of OECs are likely to result in improved axon growth. Targets for improvement can include increasing proliferation of transplanted cells; using fibroblasts to organise alignment and migration of OECs; enhancing how OECs phagocytose cellular debris; optimising the interaction between OECs and activated astrocytes to reduce glial scar formation

13.4.2 Human Trials of OEC Transplantation into the Injured Spinal Cord

Several human clinical trials have demonstrated that transplantation of OECs, of both autologous and of embryonic origin, is a safe and feasible treatment (Yan et al.

2001; Feron et al. 2005; Lima et al. 2006; Tabakow et al. 2013), even after 3 years post-implantation (Mackay-Sim et al. 2008; Huang et al. 2012). A Phase I clinical trial used autologous transplantation of OECs purified from nasal biopsies of spinal cord injury patients and showed that patients with chronic spinal cord injury did not show any adverse medical or surgical complications from the procedure, such as neuropathic pain or neurological deterioration (Feron et al. 2005; Mackay-Sim et al. 2008). In a more recent Phase I study, the transplantation of OECs together with fibroblasts was shown to be safe and feasible after 1 year (Tabakow et al. 2013). Another study showed partial recovery of bladder sensation and improvement of motor function scores in SCI patients transplanted with autologous non-purified OECs (whole olfactory mucosa grafts) (Lima et al. 2006) although this study was not conducted in accordance with Phase I or II standards and thus limited conclusions can be drawn from this study. Huang et al. (2003) reported improvement in both motor function scores and sensory perception in spinal cord injury patients aged 2–64 that received transplantation of human embryonic OECs cells, however, these results have been questioned for failing to meet safety standards for scientific protocols (Lim and Tow 2007). Encouraging results have indicated that OEC transplantation is a promising and safe option to improve recovery in human spinal cord injury patients. However, a more accurate, standardised experimental design, including appropriate controls as well as a larger number of patients, is needed in further clinical trials to confirm the feasibility and effectiveness of OEC therapy. The number of human trials conducted to date is not sufficient to conclude which degree of OEC purity is optimal. In the Phase I trial conducted by Feron et al. (2005) purified lamina propria-derived OECs from nasal biopsies were transplanted and shown to be safe, albeit no dramatic functional improvement was achieved (Feron et al. 2005; Mackay-Sim et al. 2008). Other groups (Lima et al. 2006; Chhabra et al. 2009; Aoki et al. 2010) used whole olfactory mucosa grafts (a mix of neural stem cells, OECs, fibroblasts and other associated cells) to fill the lesion cavity. In these studies some patients were reported to show improvement on motor functional and sensory neurological scores; however some adverse effects such as neuropathic pain were reported, and appropriate controls for strict comparisons were not included. The large expense and ethical concerns of conducting human trials, particularly those with large numbers of patients, confers the need to determine the optimal cell purity and cell mix to maximise the likelihood of successful outcomes. Therefore prior to conducting future human trials, it is important that the methods for purifying and identifying OECs and whether the inclusion of additional cell types is favourable should be addressed.

13.4.3 OECs are a Heterogeneous Population—Which Source Should be Used?

Within the olfactory system in vivo, OECs are not all the same, but have different characteristics and roles depending on their location in the olfactory nerve pathway. Are these different characteristics relevant for spinal repair therapies and are the

differences in the OEC subpopulations maintained after culturing and after transplantation? If so, then the source of OECs that are selected for transplantation is likely to affect the outcomes of spinal repair therapies.

OECs express a range of different molecules depending on their location in the olfactory nerve. OECs within the lamina propria express $p75^{NTR}$ and S100β which are typically used to confirm the identity and purity of OECs that have been cultured (Table 13.2). OECs within the nerve fibre layer of the olfactory bulb are not homogeneous and two OEC subpopulations are present (Fig. 13.2) which each express distinct markers. The nerve fibre layer of the olfactory bulb can be further subdivided into outer and inner layers that contain different populations of OECs with different roles in vivo. OECs of the outer nerve fibre layer are responsible for the defasciculation and sorting of axons, and inner nerve fibre layer OECs, refasciculate and target axons to glomeruli (Fig. 13.2). Expression of OEC markers also differs between the subpopulations with OECs in the outer nerve fibre layer highly expressing S100β protein and $p75^{NTR}$ (Gong et al. 1994; Au et al. 2002), whereas, OECs in the inner nerve fibre layer express low levels of S100β and $p75^{NTR}$, but do express high levels of TROY (Hisaoka et al. 2004) and NPY (Ubink and Hokfelt 2000). Therefore, based simply upon the differential expression of these markers, OECs can be considered to be a heterogeneous population of cells each with distinct characteristics that may confer different benefits for regenerating axons.

What are the proposed roles of OECs for the guidance and targeting of olfactory axons? Within the lamina propria, OECs (green in Fig. 13.2) ensheathe fascicles of axons that arise from neurons expressing different odorant receptor types. When the axon fascicles reach the outer layer of the nerve fibre layer, the OECs (red in Fig. 13.2) contribute to the defasciculation and sorting of the axons as they search for their target glomeruli. Within the inner nerve fibre layer, the OECs (blue in Fig. 13.2) contribute to the refasciculation and targeting of axons according to their odorant receptor type. Thus the OECs play different roles in these three different regions: (i) fasciculation within the lamina propria; (ii) defasciculation and sorting within the outer layer of the nerve fibre layer; (iii) refasciculation and targeting within the inner layer of the nerve fibre layer.

Consistent with their in vivo roles, in vitro studies have shown that OECs that have been purified from different regions of the olfactory system have distinctly different behaviours during cell-cell contact (Windus et al. 2007, 2010; Roloff et al. 2013). Canine OECs from the olfactory bulb have been shown to migrate faster than OECs from the lamina propria (Roloff et al. 2013). In other studies, mouse OECs from the lamina propria tend to adhere to each other, consistent with their need to promote fasciculation (Windus et al. 2007). In contrast, OECs from the nerve fibre layer of the olfactory bulb exhibit various reactions to cell-cell contact including adhesion and repulsion consistent with their roles in mediating defasciculation of axons, refasciculation and targeting (Windus et al. 2010). When olfactory sensory neurons were co-cultured with different sources of OECs, axons formed fascicle-like structures with OECs from the lamina propria whereas they tended to disperse when grown with OECs from the olfactory bulb (Windus et al. 2010). In addition, OECs derived from different regions of the nerve fibre layer (rostral, dorsal, caudal,

ventral) also exhibit varying responses (Windus et al. 2010). A lack of consistency in the purification of OECs from the olfactory bulb may account for the variation in results that are observed in the spinal transplant models. Some protocols purify OECs from the entire nerve fibre layer of the olfactory bulb (Li et al. 1997, 2007; Ramon-Cueto 2000; Lopez-Vales et al. 2006), while other protocols specify a more restricted region of the nerve fibre layer such as the rostral region (Lankford et al. 2008) or the ventral region (Guest et al. 2008). In other protocols, the region of the nerve fibre layer has not been specified (Teng et al. 2008; Torres-Espin et al. 2014) although it may be that the entire nerve fibre layer was used. Thus selecting the source of OECs could have crucial influences on the efficacy of these cells for repair of the injured spinal cord. Consistent with this, in vivo transplantation has demonstrated variable outcomes depending on the source of OECs that have been used. After transplantation into a crush injury model, OECs from the lamina propria migrated more extensively and reduced the size of the lesion cavity which resulted in more extensive axon sprouting compared to the results obtained after transplantation of OECs derived from the olfactory bulb (Richter et al. 2005). However, olfactory bulb derived OECs promoted greater angiogenesis and reduced autotomy (Richter et al. 2005). In other studies, however, the variation in outcomes depending on the source of OECs is less clear. High purity cultures of OECs from the lamina propria and from the olfactory bulb have both been shown to give similar results in reducing scar formation and promoting axon extension in comparison to control groups (Mayeur et al. 2013). If the OECs derived from the lamina propria give similar therapeutic outcomes compared to OECs derived from the olfactory bulb, then the use of the lamina propria OECs would be preferred for human therapies due to the ease of accessing the source of OECs from the olfactory mucosa.

OECs derived from the olfactory bulb offer other advantages that may make their use preferable. Typically, due to the amount of tissue that can be obtained from the nerve fibre layer, OECs from the olfactory bulb are easier to purify, result in large cell numbers, and have fewer contaminating cells such as fibroblasts (discussed below) which makes their use for animal trials attractive. Certainly, numerous studies have demonstrated improvements in anatomical or functional outcomes using OECs derived from the olfactory bulb (Table 13.2). In humans, the use of olfactory bulb derived OECs is feasible although much more invasive and primate studies have demonstrated that OECs can be successfully grown from olfactory bulbs (Rubio et al. 2008).

13.4.4 Purification of OECs and Contaminating Cells

One of the advantages of using OECs from the lamina propria for spinal repair therapies is that they can be easily harvested from the patient's own nasal mucosa. A small biopsy from the dorsal/caudal region of the nasal cavity can be obtained using local anaesthetic with minimal risk of complication. The OECs are then cultured in vitro to undergo population expansion and transplanted back into the same patient in the spinal injury site. This autologous transplantation avoids host recognition

problems however it necessitates a window of several weeks while sufficient cells are expanded in culture. Therefore early intervention to treat the spinal injury cannot occur with this method. An alternative approach is to generate a bank of purified non-autologous OECs that is stored and ready for immediate transplantation for newly injured patients. To this end, extensive work has been conducted using rodent and canine models to identify protocols that result in purification of OECs and to enhance the proliferation of the cells in vitro.

In animal models, OECs can be purified from either the olfactory mucosa lining the nasal cavity or from the nerve fibre layer of the olfactory bulb. Both of these different sources have advantages and disadvantages. Within the olfactory mucosa, the axons of the olfactory sensory neurons form large fascicles surrounded by the OECs (Fig. 13.3a). By dissecting out the mucosa, or separating the lamina propria from the overlying olfactory epithelium, the OECs can be isolated from the explant tissue. However, other cell types are also present within the lamina propria which can potentially contaminate the cultures. The accessory olfactory nerve (part of the pheromone detection system in non-human mammals) projects along the septum and is often adjacent to the fascicles of the main olfactory system (Fig. 13.3b). To date, there has been no systemic comparison of the potential contamination of OEC cultures with accessory olfactory OECs and the possible differences in their contribution to spinal cord repair.

The branches of the trigeminal nerve in which the Schwann cells are present can be in close proximity to the olfactory nerve fascicles (Fig. 13.3c). In transgenic S100ß-DsRed reporter mice (Windus et al. 2007) the different morphologies and structures of the olfactory versus the trigeminal nerves are clearly distinguishable (Fig. 13.3d–e), but the close proximity of the trigeminal nerves means that cultures of cells obtained from olfactory mucosa are likely to contain both OECs and Schwann cells unless purification techniques to remove the Schwann cells are employed. For example, in dogs, HNK-1 is a marker of Schwann cells that is not expressed by OECs and the use of magnet-activated cell sorting based on HNK-1 is effective in removing the Schwann cells to obtain populations of OECs that are free of Schwann cells (Ziege et al. 2013). However, in mice there are no such suitable differential cell surface markers that have been identified to date that can be used to separate OECs and Schwann cells effectively. Comparisons have been made between the efficacy of Schwann cells versus OECs for repair of the injured spinal cord with the results typically showing that both OECs and Schwann cells can enhance the anatomical or functional outcomes although with differences between the cell types depending on the assay (Lankford et al. 2008; Li et al. 2012; Barbour et al. 2013). While the proportion of contaminating Schwann cells is likely to be low in OEC cultures, it should still be considered that Schwann cells could be contributing to the results of spinal repair transplantation studies and may be a source of the variation in outcomes that are observed.

Fibroblasts are also present in the olfactory nerve and often contaminate OEC cultures. To date, most research efforts have attempted to purify the OECs using sedimentation techniques and complement- or antibody-mediated purification techniques with the purpose to eliminate fibroblasts. However, in many cases, partially

purified or unpurified mucosal tissues have been used with variable outcomes (Table 13.2). It has been proposed that the inclusion of fibroblasts with the OECs is essential for the functional improvements (Raisman and Li 2007) and transplantation of mixed olfactory cell populations (OECs + olfactory nerve fibroblasts) has resulted in enhanced recovery of motor function in rats when compared to OECs or fibroblasts alone, and suggested that the presence of the two cell types is necessary for optimal repair (Li et al. 2003). Recently, transplantation of semi-purified olfactory mucosa-derived cells (around 50% OECs; the remainder being fibroblasts) in dogs with spinal cord injury demonstrated that functional recovery can be achieved (Granger et al. 2012). Dogs that received the autologous cells reported a significant gain of fore-hind limb coordination as well as an improvement in the communication across the damaged segments of the spinal cord. In contrast, it has been reported that the presence of fibroblasts on olfactory mucosa cultures used for transplantation results in higher numbers of activated astrocytes, as well as an increase in inhibitory extracellular molecules that reduce axonal regeneration when compared with transplantation of pure glial cells populations (Toft et al. 2013).

To date, the roles of fibroblasts in neuronal regeneration remain relatively uncharacterised, but emerging data suggest that these cells do have crucial and active roles significantly beyond those of inactive connective tissue cells. When fibroblasts are transplanted with neural progenitor cells, they provide a scaffold that facilitates axonal re-growth after spinal cord injury (Pfeifer et al. 2004). Furthermore, fibroblasts are able to populate the lesion cavity in damaged spinal cord (Vroemen et al. 2007) and cause neural progenitor cells to differentiate into glial lineage cells (Wu et al. 2013). Thus, it is evident that a characterisation of how OECs interact with fibroblasts and regenerating neurons in a multi-cellular environment is crucial, and the optimal ratio of cells needs to be determined. For example, in peripheral nerve repair it has been shown that fibroblasts organise and promote the migration of Schwann cells (Parrinello et al. 2010), but the effect of fibroblasts on the organization and growth of OECs has not been studied extensively. There is also a question of the method used to identify OECs and fibroblasts in vitro. The marker $p75^{NTR}$ is the principal one used to identify OECs in vitro, however cultured human fibroblasts have been shown to also express $p75^{NTR}$ (Garcia-Escudero et al. 2012). This raises the question of whether the cultures of reported high purity OECs in fact contain fibroblasts and whether the therapeutic outcomes in the spinal injury models are due solely to the OECs or rather to the combination of OECs and fibroblasts.

The inclusion of fibroblasts and other cells within the "purified" OEC preparations means that the purity of the cultures varies considerably. For those experiments which have sought to use pure OEC preparations, the majority have reported the percentage of cells that express $p75^{NTR}$ as being over 90% (Table 13.2), while others have used preparations of OECs with less purity (e.g. Amemori et al. 2010; Table 13.2), but still achieve functional outcomes. Other studies have deliberately included other cells such as fibroblasts within preparations with reported purities such as 50% $p75^{NTR}$ positive cells (OECs) and 50% fibroblasts (Table 13.2). However, in a Phase I trial for humans in which the autologous purification strategies aimed to transplant high purity OECs, a lower purity was reached (76–88%; Feron

et al. 2005). In other autologous transplantation trials in which fibroblasts were deliberately included, the variation in purity of the separate cell preparations was high, with 20–50 % S100 positive cells (OECs) for a human trial (Tabakow et al. 2013) and 30–70 % for dogs (Granger et al. 2012). This indicates that the purification protocols do not result in predictable purities of cells or that the verification techniques (usually immunocytochemistry staining using antibodies against $p75^{NTR}$ and/or S100) are insufficiently accurate. For OEC transplantation to become a realistic therapy for treating the injured spinal cord, it is crucial that protocols are developed to improve the reliability of purifying, expanding and verifying the identity of cultures of OECs and/or fibroblasts.

13.5 Limitations of the Use of OECs

One of the disadvantages of using OECs for transplantation is the limited survival and low proliferation rates observed over time in culture (Doucette 2002), making large-scale expansion of OECs a main challenge. Moreover, changes in the local environment may induce changes in the morphology of OECs, affecting their behaviour (Vincent et al. 2005a) and causing cells to exhibit characteristics associated with specific roles in nerve regeneration (Doucette 1995; Woodhall et al. 2003).

OECs display a range of phenotypes in vitro and in vivo, and they are able to transform from one morphology type to another in response to modifications in the culture conditions (Doucette 1995; Windus et al. 2007, 2010; Huang et al. 2008a; Radtke et al. 2010). In culture, OECs display different morphologies, including a flattened sheet-like morphology (astrocyte-like), bipolar fusiform morphology (Schwann cell-like) and multipolar morphology (Chuah and Au 1994; Huang et al. 2008b). Morphology of OECs is known to be affected by variables as the method of isolation and purification, cell culture conditions as well as presence of extracellular and intracellular molecules such as cAMP, dBcAMP endothelin-1 and fibulin-3 (Vincent et al. 2003; Huang et al. 2008b; Vukovic et al. 2009). For example, expression of fibulin-3 in OECs results in a predominant bipolar morphology with extremely long processes (Vukovic et al. 2009), whereas local changes in cAMP levels lead to dynamic morphology alterations dramatically affecting OECs migration rate (Huang et al. 2008b).

Studies have shown that OEC plasticity is more evident after cells have been exposed to activated astrocytes and inflammatory mediators within a neuronal lesion site (Woodhall et al. 2003). This extreme plasticity in morphology and physiological changes in populations of OECs before and after transplantation may be a further cause for the discrepancy of results between studies. Thus, it is necessary to develop an optimal standard method by which OECs can be effectively propagated whilst maintaining a consistent or predictable phenotype. At the very least, a thorough characterisation of the mechanisms involved in regulating OEC morphology and behaviour is critical to guarantee better outcomes for OEC transplantation trials.

Another concern presented in transplantation of glial cells as a therapy for spinal cord injury is the inability of transplanted cells to integrate in the host spinal cord. This is due to the over-expression of proteins that inhibit migration, such as Nogo, known to alter the morphology of lamellipodial membrane protrusions in OECs to favor focal adhesion rather than migration (Xu et al. 2009). Some molecules such as proteoglycans, can block the production of these inhibitory proteins, thus promoting the migration of transplanted cells into injured spinal tracts (Huang et al. 2008a). In an olfactory injury model, it has been demonstrated that OECs migrate more extensively without the presence of axons, but that subsequent axon growth is then superior (Chehrehasa et al. 2010). After transplantation, OECs migrate ahead of regenerating axons through unfavorable growth environments increasing the potential of damaged axons to extend through the damaged site (Ramon-Cueto and Santos-Benito 2001; Chehrehasa et al. 2010; Ramon-Cueto 2011). Identification of factors or small molecules that enhance OEC migration properties could offer new alternatives for develop further strategies with emphasis in combinations of cell transplantation and drug therapy that could increase the efficacy of actual OECs transplantation treatments. For example, as described above and has been tested in vivo, OECs could be genetically engineered to express growth factors that would promote their function (Cao et al. 2004; Wu et al. 2008).

13.6 Future Outlook and Opportunities

The use of combination therapies involving OEC transplantation and neural growth factors or molecules that mimic their neurotrophic effect are likely to achieve better results than conventional therapies, and provide a more efficient solution for neural repair in humans (Doucette 1995). Neuregulins and other neurotrophic factors are involved in the survival, proliferation and migration of OECs, as well as Schwann cells, oligodendrocytes and astrocytes. All different types of glial cells present receptors for neuregulins, molecules that promote glial growth and regulate the role of glial cells on nerve regeneration. OECs express various neuregulins including Neu differentiation factor, glial growth factor and motor-neuron derived factor, as well as many receptors for these molecules, supporting the theory that OECs play an important role in the survival, growth-promotion and regeneration of severed nerve cells (Woodhall et al. 2001, 2003; Vincent et al. 2005a). An increased understanding of how glial cells respond to neurotrophic molecules as well as the identification of novel compounds that enhance the migratory and integrative properties of OECs is therefore highly warranted in the field.

Another approach is the use of biomaterials and tissue engineering strategies that facilitate axon growth in the spinal cord, as well as to enhance the survival and integration of OECs or other cells transplanted to the injury site. Extracellular matrix motifs and bio-scaffolds can provide the transplanted cells with an enriched substrate that may increase cell proliferation and migration. For example, matrix

proteins such as fibronectin and laminin, used on matrix-based scaffold have anti-apoptotic properties that may lead to an improvement on cell survival and migration (Friedman et al. 2002b, a).

To date, experimental and clinical studies suggest that OEC transplantation is safe and effective and may provide real benefits in the treatment of spinal cord injuries. However, it is important that a better understanding of the biology of OECs be pursued to enable new strategies to be developed, perhaps with a more multifaceted approach, to achieve an effective use of OECs in the treatment of spinal cord injury in humans.

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