AMERICAN JOURNAL OF MEDICAL AND DENTAL SCIENCES
ISSN Print: 2326-8638, ISSN Online: 2326-8648, doi:10.5251/ajmds.2013.1.1.21.24
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A preclinical update on the utility of olfactory ensheathing glia and embryonic stem cells as cellular therapies for spinal cord injury.

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ABSTRACT

Cellular therapies offer a novel facet for the management of spinal cord injury based on regenerative approaches, which has shifted the treatment paradigm away from rehabilitative, palliative and symptomatic treatments towards the potential attainment for complete functional recovery. This review critically considers the impact of recent preclinical and scientific evidence on the utility of olfactory ensheathing glia and embryonic stem cells in the management of spinal cord injury.

Keywords: Spinal Cord Injury; Embryonic Stem Cell; Olfactory Ensheathing Glia; Regeneration; Functional Recovery

INTRODUCTION

Spinal Cord Injury (SCI), which refers to damage to the spinal cord, is associated with significant handicap, disability and impairment partly because current licensed treatments are limited in their application to rehabilitative and palliative care. The attainment of complete functional recovery is hindered by multiple challenges due to the primary injury with secondary damage, which causes neuronal loss, demyelination and axonal disruption that cumulatively leads to cystic degeneration and an inhibitory astroglial scar.

Novel treatment paradigms have challenged the traditional gloomy impression that transected axons are incapable of regeneration and have honed in on the utility of cellular therapies, particularly the role of Olfactory Ensheathing Glia (OEG) and Embryonic Stem (ES) cells.

OEG are glial cells located within the olfactory epithelium, olfactory nerve, and the outer two layers of the olfactory bulb that function synergistically with Schwann cells to create a 3-dimensional matrix, which provides a permissive microenvironment that *indirectly* promotes axonal regeneration [1]. In contrast to and distinct from OEG, ES-cells are primitive, undifferentiated, pluripotent cells derived from the inner cell mass of the blastocyst, which are capable of self-renewal.

Based on the functional regenerative potential of OEG and ES-cells, this review will critically consider the evidence basis underlying the efficacy of these specific cellular therapies in the treatment of SCI.

OEG and ES-cells promote functional recovery in SCI through: bridging cavities and cysts; regenerating damaged neuronal and glial cells; creating a permissive microenvironment that promotes axonal regeneration; and via modulating intact and relay circuitry. The scientific and preclinical evidence underlying these mechanisms will be discussed in turn.

In vitro OEG have been shown to support axonal growth via upregulation of the low-affinity NGF p75 receptor and the secretion of neurotrophic growth factors, which facilitates remyelination, extension and elongation in anatomically preserved but unmyelinated axons [2]. These findings have been extrapolated to *in vivo* transplantation experiments in rodent and non-human primate models of SCI, which demonstrated that OEG: integrated into the host spinal cord with negligible astrogliosis [3]; supported axonal re-myelination [4]; guarded against secondary damage and neural cavitation [5]; enhanced

angiogenesis at the lesion site [6]; promoted neurite outgrowth and path-finding of adjacent spared axons [7]; and facilitated extensive axonal migration throughout the entire spinal cord [8].

However, these observations need to be proportionally assessed against the limitations on the utility of OEG, which include: expressing inhibitory molecules [9]; susceptibility to viral infections [10]; producing lower levels of neurotrophic growth factors relative to Schwann cells [11]; and inconclusive and non-reproducible evidence to promote axonal regeneration. On this point there is a contentious body of evidence against the myelinating capacity of OEG, which stemmed from comparative experiments on the efficacy of Schwann cells and OEG to myelinate neurites sprouting from dorsal root ganglia in culture [12]. However, the findings of these experiments are limited in their application for two reasons: the OEG used were selected from a sub-population of NGF p75 positive cells that may not have the same myelinating capabilities relative to NGF p75 negative cells; and adult rat OEG harvests were used, which have an inferior myelinating potential compared to embryonic OEG harvests.

Similar to OEG, there is an emerging body of preclinical and scientific data on the utility of ES cell-derived neural cells to bridge cavities and cysts in SCI. As alluded to earlier, the secondary damage from SCI is associated with apoptosis of myelin producing oligodendrocytes with subsequent demyelination. In a rodent model of SCI transplanted ES-cells that were pre-differentiated into oligodendrocyte progenitor cells (OPCs) re-myelinated spared axons with demonstrable functional recovery [13]. Disadvantages included: difficulty in engineering high-purity lineage-specific cell lines without karyotypic abnormalities; ethical issues relating to disaggregation of the developing blastocyst; and safety concerns regarding tumorigenesis.

In addition to bridging spinal cavities and cysts, evidence suggests that OEG may have a role in regenerating damaged neuronal and glial cells in SCI. A study illustrated that transplantation of OEG into a dorsal hemisection spinal cord resulted in the formation of ‘cellular tunnels’ across the transection site enabling axons to regenerate through [14]. Based on this observation it was hypothesised that two phenotypes of OEG may exist: ‘*A*’ cells, which form the tunnels; and ‘*S*’ cells, which myelinate regenerated axons within the cellular tunnels [15].

Leading on from this, evidence from a rodent model of complete SCI demonstrated that OEG assisted the regrowth of nerves across the injury site with resultant functional behavioural recovery including walking, climbing, reaching and breathing [16]. However, the OEG used in that experiment were derived from the olfactory bulb, which is a poorly accessible and impractical site for biopsy relative to the olfactory mucosa. Improved OEG harvesting methods would be needed if these findings are to be extrapolated into large scale clinical trials.

ES-cells have also been used to regenerate the cytoarchitecture in SCI. Xenograft transplantation experiments on human ES cell-derived neural precursor cells in collagen scaffolds into rodent models of cervical SCI were found to improve hindlimb sensorimotor function. The grafted cells migrated towards the site of SCI with demonstrable neuronal and glial differentiation [17]. However, these results provide no information on the utility of ES-cells in thoracic SCI where larger scale graft migration would be needed to achieve the same.

OEG may foster functional recovery in SCI by collaborating with other cell types to create a permissive environment that facilitates axonal regeneration. Evidence suggests that OEG collaborate with host Schwann cells to create a 3-dimensional matrix containing extracellular matrix molecules, growth factors and transcription factors, which collectively function as a substrate conducive to the regrowth and remyelination of injured axons [18]. Furthermore, by intermingling with astrocyte cell processes, OEG impede astrocyte-mediated chondroitin sulphate proteoglycan up-regulation thus forming a neuroprotective barrier between host Schwann cells and the hostile environment within the damaged spinal cord [19]. *In vitro* OEG promoted Schwann cell migration to the lesioned site by releasing chemotactic factors, such as NGF-74 and NGF-75, which bind to the Schwann cell p75 NTR [20]. These *in vitro* experiments were extrapolated to an *in vivo* study, which found that labelled OEG supported functional recovery in SCI by providing a favourable chemotactic environment that stimulated the migration of Schwann cells into the damaged spinal cord [21].

Limitations on the evidence regarding the utility of OEG to create a permissive environment, trophic support, and neuroprotection conducive to functional recovery in SCI include uncertainty regarding the long-term fate of transplanted OEG. Furthermore, despite OEG integrating within the lesioned spinal

cord and robustly associating with myelinating axons *in vivo,* it is unknown whether these processes contributed to the observed functional recovery.

Evidence also supports the efficacy of transplanted ES-cells to promote functional recovery by modulating the microenvironment in SCI. To illustrate this principle, ES cells have been shown to: promote oligodendroglial differentiation as an effective strategy to remyelinate damaged axons [22]; and contribute to promoting axonal regeneration by functioning as cellular scaffolds for growing axons [23]. Disadvantages of using ES-cells in this regard include overcoming the effects of the hostile environment in SCI that promotes astrocytic- over oligodendrocytic- differentiation with resultant scar formation rather than remyelination.

Preclinical evidence using electrophysiological and anatomical assessments suggest that OEG may promote functional recovery in SCI by modulating intact and relay circuitry. An *in vivo* study [24] in rodents found that seven months after complete spinal cord transection 70% of OEG-transplanted rats demonstrated motor-evoked potentials in hindlimb musculature after transcranial electrical stimulation. This axonal regeneration was further tested by re-transection, which suppressed motor performance and reduced the hypersensitive hindlimb withdrawal response to mechanical stimulation. These results suggested OEG transplantation supported axonal regeneration across the transection and reorganisation of spinal circuitry. Limitations of the study included a failure to perform detailed sensory testing and discrepancies between anatomical and electrophysiological results.

Evidence also supports the role of ES-cells in this regard; graft-derived neurons were shown to re-establish neuronal relays and connectivity between injured dorsal column sensory axons and denervated dorsal column nuclei [25]. However, the fidelity of the relays was compromised by partial failure of neurons to navigate to correct targets.

In conclusion there is a wealth of preclinical evidence on the utility of OEG and ES-cells to promote functional recovery in SCI. Further research is needed to conclusively establish whether OEG promote plasticity, regeneration, remyelination, or neuroprotection. Determining the optimal source and age of OEG for transplantation, the best graft strategy, and the correct timing of transplantation may turn the hope of these therapies into expectation.

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