The link between olfactory ensheathing cell survival and spinal cord injury repair: a commentary on common limitations of contemporary research

PERSPECTIVE

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Olfactory ensheathing cells (OECs) are crucial players in the continuous regeneration of the olfactory nervous system that occurs through out life and are thought to have unique growth-promoting properties. For this reason, OEC transplantation has been thoroughly explored for the potential to promote neural repair after both central and peripheral nervous system injuries. Numerous studies have shown that OEC transplantation is safe and can promote recovery after spinal cord injury (SCI), both in animal models and in human clinical trials. To date, a variety of injury types and time-points after injury, as well as different delivery methods, have been tested. Outcomes have been encouraging (in rodent models including, for example, restoration of locomotion, breathing and climbing ability along with induction of axonal sprouting and some axonal regeneration) but highly variable (Barnett and Riddell, 2007; Gomez et al., 2018). In their natural environment of the primary olfactory nervous system (the olfactory nerve and outer layer of the olfactory bulb), OECs provide structural support for olfactory axons and secrete a range of growth and guidance factors as well as basement membrane components. OECs also phagocytose debris arising from degenerating axons (Ekberg and St John, 2014). In the injured spinal cord, OECs (in addition to these functions) also exhibit a unique capacity for migration into scar tissue and for integration with astrocytes (Barnett and Riddell, 2007; Gomez et al., 2018). For these neural repair effects to occur, it is essential that the transplanted cells survive over time. The key factor for success is thus that the OECs must not only arrive at the right place within the injury site, but must also over time integrate and interact with the injured tissue.

To date, many studies do not report on OEC survival and it is thus not well known how many of the transplanted cells survive over time. A recent review (Reshamwala et al., 2019) focused specifically on OEC survival after transplantation in rodent models of SCI over the last 10 years, constituting the first published review article that specifically addresses the link between cell survival and SCI repair. The review analyzed how different studies have determined cell survival, assessed the methodologies used throughout the studies (injury model, method of cell delivery, identification of OECs after transplantation) as well as the interrelationship between cell survival and functional/structural outcomes. The review confirmed that cell survival has not often been discussed or quantified in the published animal trials; the reason being that it is difficult to track the cells after transplantation. OECs do not express any characterized cell-specific markers that definitively identify them from other glial cells. Thus, establishment of a panel of markers labeling OECs is essential.

In the studies that did assess OEC survival, the survival rates were in general low (less than 3% after 3–4 weeks with the highest reported being 6.5 ± 2.5% at 4 weeks; some studies reported ~20% survival in areas distant from the injury site). The review identified several key factors that influenced cell survival and integration. These included (1) the injury model used, (2) the anatomical source of OECs (olfactory mucosa/nerve *versus* bulb), (3) whether cells were co-transplanted with other cell types, (4) the number/concentration of transplanted cells, (5) method of transplantation and (6) the time between injury and transplantation (discussed in more detail below).

Injury model–animal and type of injury: Transection injury typically induces less inflammation within the injury site than contusion/crush injuries. Therefore, the millieu at the transection injury site has been suggested to be less hostile to the transplanted cells than that at a contusion/crush injury site (Reshamwala et al., 2019). A recent study assessing transplantation of fetal brainstem cells, however, suggests that survival of transplanted cells (estimated as % area of green fluorescent protein-expressing cells in the injury site) may be better in crush-type than transection-type injury (Hou et al., 2018). To date, most studies in rodents have been using transection-type injury, so a definite conclusion of differences in cell survival between the two models can not be made (due to an insufficient number of crush/contusion studies). From a translational perspective, it is essential that more studies use crush- or contusion models since such injuries are much more frequently encountered clinically.

Source of OECs–olfactory mucosa or bulb: A biopsy containing OECs can be taken either from the olfactory mucosa lining the nasal cavity (which contains olfactory nerve fascicles) or from the olfactory bulb within the cranial cavity (**Figure 1**). Both sources have been used in animal models and human clinical trials; in humans, OECs are typically transplanted as autografts (donor and recipient are the same person), which eliminates the need for immunosuppressants. As the olfactory bulb is part of the brain, bulbar biopsies require intracranial surgery and removal of brain tissue. Thus, mucosal biopsies are highly favourable as they do not require invasive surgery. To date, most animal studies have used bulbar OECs as they tend to generate higher purity cell preparations, but as cell survival has not been tracked in many animal studies there is no clear evidence advantages of using bulbar OECs in terms of cell survival or structural/functional outcomes. Therefore, it would be advantageous if more animal model studies in the future could be focused on using the more clinically relevant mucosa-derived OECs.

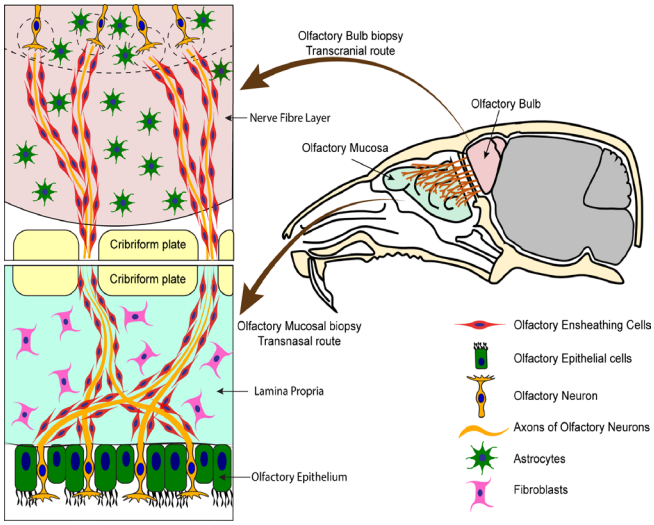

Figure 1 Olfactory ensheathing cells (OECs) can be obtained from either the olfactory mucosa lining the upper nasal epithelium, or the olfactory bulb within the cranial cavity of rodents.
OECs can be obtained from the same sites in humans. The olfactory mucosa is the preferred source as it is easily accessible in humans, however the numerous cell types can lead to cultures of lower purity. In contrast, the olfactory bulb is a rich source of OECs with fewer contaminating cells, but requires invasive surgery that can lead to permanent loss of the sense of smell and is therefore not a suitable source for human therapies.

Co-transplantation with other cell types: OEC cultures often contain additional cell types which may or may not affect outcomes. In general, many studies have not shown better survival when OECs are co-transplanted with other cell types (Reshamwala et al., 2019). Several studies using different types of stem cells, however, suggested that co-transplantation with OECs improved stem cell survival, as well as structural or functional outcomes.

Cell number and concentration: The number or concentration of transplanted cells also constitute a key factor determining cell survival. In most animal studies, 100,000–500,000 cells were transplanted. When this number was drastically reduced, or when the concentration of cells was very low, survival was impaired (Reshamwala et al., 2019). This is likely because OECs require cell-cell contact for survival and for many of their functions. Conversely, very high cell numbers or cell densities may lead to increased shear stress or physical damage during transplantation and thus poor survival. Very high cell numbers can also prove problematic due to the larger treatment volume required to accommodate the cells, and the presence of numerous dead cells in the injury site could have

direct detrimental effects. Another review (Watzlawick et al., 2016) has identified that a range of 150,000–180,000 cells has been associated with the most optimal effect size.

Method (mode) of transplantation: Most studies to date have used intra-spinal injections of cells in suspension, which has limitations in terms of the volume of treatment and the handling of the cell preparation. For this reason, the review suggested that transplanting the cells in a three-dimensional (3D) construct may help overcome the volume barrier and enable successful transplantation of high cell numbers without detrimental effects. Being in a 3D construct also allows the cells to form an integrated network prior to transplantation, likely not only improving cell survival but also many OEC functions such as contact-dependent migration (Windus et al., 2007). A 3D construct may also offer additional advantages over conventional suspension injections such as easy handling and better control over direct transplantation into the injury site.

Time between injury and treatment: The inflammatory state of a SCI site changes dramatically over time. Thus, the duration between injury and cell transplantation is likely to affect survival of the transplanted OECs. Most transplantation studies to date have focused on the acute phase with few studies focusing on the sub-acute or chronic phases; thus it is difficult to compare cell survival rates between the acute phase and the sub-acute or chronic phase. The transplantation of cells in the sub-acute phase may be beneficial (after acute inflammation but before onset of chronic inflammation) and more studies need to focus on sub-acute or chronic SCI, as OEC transplantation in humans immediately following the injury may not be either logistically viable nor medically advisable. In addition, an important avenue for improvement of cell survival can be modulation of the injury site in the acute, sub-acute or chronic phase by management of the inflammation. It has been well documented that immunosuppressants, whilst not needed to counteract graft rejection of autologous transplants, may have beneficial effects on the SCI site. A recent study suggested that transient immunosuppression may provide enough opportunity for transplanted OECs to survive, integrate and induce repairs (Li et al., 2016).

Link between SCI repair and OEC survival: A definite correlation between OEC survival rate and functional outcomes cannot be drawn, because so few studies have quantified the number of surviving cells; improving our understanding of this important area may lead to enhanced outcomes in future. A few studies, however, have linked poor functional or structural outcomes to low cell survival (Pearse et al., 2007; Novikova et al., 2011). One study noted that micro-implantation of cells can enhance their survival and thus efficacy in a contusion injury model (Pearse et al., 2007). The same study also noted that key factors affecting cell survival and integration (such as cell density, transplantation location and mode of delivery) need to be properly optimized to ensure axonal extension across the injury site in the desired rostro-caudal direction.

Conclusion and other key issues: A recent review focused on the important issue of OEC survival after transplantation in rodent models of SCI (Reshamwala et al., 2019). However, other key areas to be considered include the level of SCI and the size/severity of the injury, which may both affect survival of the transplanted cells. The inflammatory state of the SCI, which can affect cell survival, can vary significantly with both level of injury and injury severity (Hong et al., 2018). To date, conclusions regarding the effects of injury level/severity on cell survival cannot be made since very few studies have compared the effects of these factors on transplantation outcomes. Another key issue not covered by the review is the fact that culture conditions and time in culture prior to transplantation can affect the long-term survival and phenotype of OECs (Liadi et al., 2018). Again, insufficient amounts of comparative literature and high variability between culture conditions in different studies makes it difficult to conclude how *in vitro* culture prior to transplantation affect survival of cells after transplantation.

Overall, survival of OECs after transplantation into the injured spinal cord has been poorly described in the literature as tracking of transplanted cells is difficult. Therefore, reliable methods for identifying transplanted cells need to be established, particularly OEC marker panels. It is likely that the transplantation of cells in 3D constructs may significantly improve OEC survival and the advent of 3D cell culture technologies may assist with the design of appropriate cell preparations for transplantation. Despite the variability in the outcomes, OECs show significant potential to induce structural and functional recovery in the injured spinal cord. It is hoped that future studies will seek various avenues to improve cell survival, and the reporting of cell survival, which will lead to enhanced outcomes.

This work was supported by a Clem Jones Foundation grant to JS and JE, a Perry Cross Spinal Research Foundation grant to JE and JS, a Motor Accident Insurance Commission grant to JS and JE, and a Griffith University International Postgraduate Research Scholarship to RR.

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***Received:** *December 17, 2019*
***Peer review started:** *December 23, 2019*
***Accepted:** *January 21, 2020*
***Published online:** *April 3, 2020*

***doi:** *10.4103/1673-5374.280310*

***Plagiarism check:** *Checked twice by iThenticate.*
***Peer review:** *Externally peer reviewed.*

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C-Editors: Zhao M, Li JY; T-Editor: Jia Y