PERSPECTIVE

Tayloring cell populations for neurodegenerative diseases

Neurological disorders are increasing in prevalence worldwide, and interest in stem cell therapies for these afflictions has increased over the past two decades. While many neurological injuries are too devastating for the repair capabilities of endogenous neural stem cells (NSCs) an alternative is to harvest stem cells from a donor and grow them *in vitro*, to be used later as a donor source for transplantation. Many research groups have already done this, first using animal models and now using clinical trial participants. Despite the regular flow of publications about cell replacement therapies for central nervous system (CNS) disorders, there is still a scarcity of clinically-relevant reports of efficacy. The capability of donor cells to undergo ample site-directed differentiation and functional integration seems to be lacking (Andressen, 2013). So, while stem cells do have properties that are suited for repair of the injured CNS, a primary remaining question is how these cells can best be grafted to produce long-term functional benefit to the host environment. Moreover, among the challenges in neural cell transplantation is controlling the ultimate characteristics of grafted cells, pertaining to their survival, phenotypes and performance.

On the subject of candidate cell sources for neuro-transplantation, clinical trials have already utilized progenitor cells harvested from multiple different first-trimester fetal CNS regions. Fetal human neural precursor cells (hNPCs), in culture, can respond to mitogens and give rise to mitogen-responsive progeny in large numbers. Subsequent differentiation into the major CNS cell types is achievable upon mitogen withdrawal and application of appropriate signaling molecules, such as brain-derived neurotrophic factor (BDNF), bone morphogenetic protein 4 (BMP4) or platelet-derived growth factor (PDGF). Fetal hNPCs, as well as adult-derived hNPCs, are multipotent (*i.e.*, they are more restricted than embryonic stem cells in the lineages they can generate). Our group has presented data demonstrating the multipotency of fetal hNPCs (*i.e.*, their tripotent ability to differentiate into neurons, astrocytes and oligodendrocytes) (Fortin et al., 2016), which have precedent in the literature as being able to survive, mature into neuronal cell types and integrate functionally upon transplantation (Piroth et al., 2014). However, transplanting uncommitted cell types is problematic because this allows little control over the true composition of the therapeutic dose. Uncommitted cells, despite possessing the potential of choosing a neuronal fate, may predominantly choose a glial fate, in addition to carrying the threat of proliferation (Amariglio et al., 2009). Moreover, since donor cells routinely have a high mortality rate upon implant (Sato et al., 2008), it becomes all the more important to ensure that the original cellular composition is relatively homogenous and committed towards the appropriate fate. With these factors in mind, we designed a method of purifying the differentiated cell types before grafting (Azari et al., 2011; Fortin et al., 2016).

The technology we described seeks to optimize the composition (*i.e.*, phenotypic distribution) of transplanted cells, with consideration to injury type and injury stage, for effectively protecting endogenous neurons and replacing those already lost. Depending on the time elapsed since injury, the appropriate objective of therapeutic intervention may be quite different. In the case of stroke, for example, early transplants post-insult may best be designed to rescue host tissue from its own hostile milieu. The acute phase is characterized by an excitotoxic, inflammatory host environment fraught with mitochondrial failure and high free radical levels (Bennet et al., 2012). Providing an astrocyte-rich cellular implant at this time is anticipated to abate this process. Previous research has indicated the role of astrocytes in reducing both inflammation and excitotoxicity in the host setting (Bennet et al., 2012), as well as in enhancing neuronal survival and integration (Barde, 1989). However, around 5 days post-insult, it may be appropriate to transplant with the intention of actually repairing and replacing tissue damaged during the earlier stages. Furthermore, when transplanting predominantly immature neurons, the inclusion of 10–20% astrocytes may improve the survival and neurite outgrowth of the overall implant (Barde, 1989). Providing an immature neuron-*rich* population, in combination with astrocytes, is anticipated to preserve host neuronal networks and introduce the necessary trophic factors. Indeed, an important benefit of pre-defining the cellular dose is the ability it gives researchers to alter the combination of cell types in pursuit of a precise treatment.

In previously published work, we used an *in vitro* protocol to induce neurogenesis in hNPCs, followed by a simple cell-type separation technique, and demonstrated that pre-defining the maturity and phenotype distribution of a cellular implant leads to a degree of predictability over donor cell phenotypic fate *in vivo* (Fortin et al., 2016). The *in vitro* protocol we used to induce neurogenesis was the neuroblast assay (NBA)(Azari et al., 2011). Briefly, the NBA uses poly-ornithine and laminin to induce an adherent monolayer culture, with BDNF to encourage neuronal differentiation. After six days in the NBA, immature neurons are isolated from glia by first immuno-labeling the extracellular PSA-NCAM antigen and then performing the immunomagnetic cell purification technique known as magnetic activated cell sorting (MACS). This work was intended to address the challenges of grafted-cell phenotypic

fate and survival by pre-differentiating hNPCs *in vitro* and then transplanting a defined population. Would we yield more neurons and/or fewer astrocytes *in vivo* after implanting a population that was pre-enriched for neuronal hNPC progeny? We sought to give proof of principle that transplant tissue can be engineered *in vitro* for phenotypic distribution and maturity, and that such engineering can result in foreseeable *in vivo* graft characteristics. A potential pitfall associated with grafting post-mitotic cells, which may be therefore less resilient, is the risk of poor donor cell survival. As cells are differentiated and matured *in vitro* they may become less versatile and the overall graft may become heavily subject to cell death. However, if the goal of a cell graft is, for example, to replace damaged neurons, then implanting a high concentration of neurons is potentially more valuable than implanting a low concentration of neurons, even if the latter graft survives in higher number.

Using a high percentage of immature human neurons, we established that these neurons can survive as well as their multipotent progenitors upon grafting in the mouse brain. The purified neuronal graft also generated fewer astrocytes *in vivo* than the undifferentiated graft, because the former cells were first raised *in vitro* under conditions supporting neurogenesis, decreasing the cells' phenotypic versatility. Because fewer glia were produced, potential problems associated with excess astrocytes, such as allodynia, could be prevented using this methodology. Generating fewer glia and more neurons from a graft may also be vital for circumstances that particularly benefit from neuronal *replacement or supplementation*, such as in the chronic phases of stroke (Hao et al., 2014) or in temporal lobe epilepsy (Cunningham et al., 2014), respectively. On the other hand, the pre-differentiation and purification system discussed herein may still hold promise in contexts of more acute injuries, where an astrocyte-*rich* graft may provide trophic factors and reduce glutamate and free radicals.

As cell therapy advances toward clinical application, more precision is urgently needed. The hNPCs that we culture and have used in published experiments are proliferatively and neurogenically reliable in long-term tissue culture, generating neurons and astrocytes that can be purified *in vitro*. Upon transplantation, purified hNPC-derived neurons resulted in fewer astrocytes two months post transplant, as compared against heterogeneous hNPCs. The transplant model discussed here may lower the risks of uncontrolled donor-cell phenotypic fates, including long-term tumorigenesis (Amariglio et al., 2009) and excessive astrocyte differentiation, and may augment survival of preferred cell types. The investigative power obtainable through grafting defined cell populations may help researchers examine the influence of different, uniquely-defined cell grafts on specific injury environments.

Jeff M. Fortin, Loic P. Deleyrolle, Brent A. Reynolds*

Lillian S. Wells Department of Neurosurgery, University of Florida, Gainesville, FL, USA (Fortin JM, Deleyrolle LP, Reynolds BA)
Preston A. Wells, Jr. for Brain Tumor Therapy, University of Florida, Gainesville, FL, USA (Deleyrolle LP, Reynolds BA)

***Correspondence to:** Brent A. Reynolds, Ph.D., b.a.reynolds@gmail.com.*
***Accepted:** 2016-10-08*
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