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Primary tissue for cellular brain repair in Parkinson's disease: Promise, problems and the potential of biomaterials

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Abstract

The dopamine precursor, levodopa, remains the 'gold-standard' treatment for Parkinson's disease, and, although it provides superlative efficacy in the early stages of the disease, its long-term use is limited by the development of severe motor side effects and a significant abating of therapeutic efficacy. Therefore, there remains a major unmet clinical need for the development of effective neuroprotective, neurorestorative or neuroreparatory therapies for this condition. The relatively selective loss of dopaminergic neurons from the nigrostriatal pathway makes Parkinson's disease an ideal candidate for reparative cell therapies wherein the dopaminergic neurons that are lost in the condition are replaced through direct cell transplantation into the brain. To date, this approach has been developed, validated and clinically-assessed using dopamine neuron-rich fetal ventral mesencephalon grafts which have been shown to survive and re-innervate the denervated brain after transplantation, and to restore motor function. However, despite long-term symptomatic relief in some patients, significant limitations, including poor graft survival and the impact this has on the number of fetal donors required, have prevented this therapy being more widely adopted as a restorative approach for Parkinson's disease. Injectable biomaterial scaffolds have the potential to improve the delivery, engraftment and survival of these grafts in the brain through provision of a supportive microenvironment for cell adhesion, growth and immune shielding. This article will briefly review the development of primary cell therapies for brain repair in Parkinson's disease, and will consider the emerging literature which highlights the potential of using injectable biomaterial hydrogels in this context.

Introduction

Cell replacement therapy for neurodegenerative diseases has emerged from a relatively simple conceptual framework - if the primary pathological feature of a disorder is the degeneration and subsequent loss of a specific neuronal subtype, then the replacement of these cells should repair the brain and restore function to the patient. The relatively selective loss of dopaminergic neurons from the substantia nigra makes Parkinson's disease an ideal candidate for cell replacement therapy (Fearnley *et al.*, 1991). To date, cellular brain repair for Parkinson's disease has been developed using tissue dissected from the embryonic ventral mesencephalon (VM) which incorporates the developing dopaminergic neurons from the substantia nigra and ventral tegmental area. As this is the current "gold standard" source of dopaminergic neurons for brain repair in Parkinson's disease, this will be the focus of this review. However, as it will be essential in the future to move towards a more standardised cell source (such as neurons derived from pluripotent stem cells), the reader is also directed to the article by Malin Parmar and colleagues in this Special Issue for a review of developments in stem cell-derived dopaminergic neurons for brain repair in Parkinson's disease.

Cellular brain repair for Parkinson's disease – Preclinical studies

The transplantation of primary dopaminergic neurons began in the 1970s when Björklund and colleagues transplanted small pieces of tissue (containing dopaminergic, serotonergic and noradrenergic neurons) dissected from the fetal rat brain into the cerebral cortex and hippocampus of unlesioned adult rats. These cells showed good survival after transplantation with substantial axonal outgrowth that formed extensive fiber patterns within the graft itself and with adjacent brain tissue (Bjorklund *et al.*, 1976; Stenevi *et al.*, 1976). Then, in unilateral rodent models of Parkinson's disease, Perlow *et al.* (1979) transplanted solid tissue pieces into the lateral ventricle in contact with the caudate-putamen, while Bjorklund *et al.* (1980) transplanted tissue pieces to the dorsal cortical cavity overlying the caudate-putamen. Both studies noted improvement in amphetamine-induced rotational behavior, and *post mortem* analysis revealed survival and re-innervation of the lesioned striatum. A setback to these original studies was the use of pieces of dissected brain tissue, necessitating the need to create a highly vascularized cavity within the brain, while also preventing the targeting of deep brain structures (Dunnett *et al.*, 1981). This led to the generation and use of dissociated cell suspensions, which allowed for cell transplantation at multiple sites of the striatum, resulting in more widespread innervation and better functional recovery in rotational behavior as well as sensorimotor tests (Bjorklund *et al.*, 1980; Bjorklund *et al.*, 1983a; Schmidt *et al.*, 1983). Interestingly, it was shown that functional recovery caused by the transplantation of

fetal tissue was specific to cells derived from the dopamine-rich VM. This confirmed that the effects seen were dependent on dopamine replacement in the striatum and not on non-specific stimulation by the fetal tissue (Dunnett *et al.*, 1988). From here, the field of cell replacement therapy in Parkinson's disease rapidly expanded.

It is now well established that the efficacy of cell replacement strategies in Parkinson's disease is dependent on 1) the survival and maturation of dopaminergic neurons in the host brain, 2) appropriate axonal outgrowth from the transplanted cells, 3) integration with the host system and 4) restoration of dopamine transmission. Numerous studies have shown that dopaminergic neurons from the developing VM can mature and function in the host adult striatum following transplantation (Annett *et al.*, 1997; Brundin *et al.*, 1987; Dowd *et al.*, 2004; Hahn *et al.*, 2009; Kauhausen *et al.*, 2013; Parish *et al.*, 2008; Torres *et al.*, 2008). Additionally, electrophysiological and neurochemical studies have shown that grafted dopaminergic neurons are capable of the synthesis, release and uptake of dopamine (Rose *et al.*, 1985; Schmidt *et al.*, 1982; Zetterström *et al.*, 1986), exerting electrical firing patterns (Wuerthele *et al.*, 1981), re-innervating the host striatum and developing graft-to-host synaptic connections (Bolam *et al.*, 1987; Mahalik *et al.*, 1985). Moreover, dopamine release from transplanted neurons in the striatum can restore basal dopamine levels towards normal (Piccini *et al.*, 1999). Overall, while transplanted dopaminergic neurons are capable of survival and extensive afferent and efferent connectivity with the host brain, it must be noted that there are a number of factors which can affect the efficacy of VM transplantation including graft survival, placement, neuronal subtype and donor age.

Impact of graft placement

Preliminary *in vivo* studies have given significant insight into how the specific placement of fetal VM grafts in the brain can affect the efficacy of transplantation. In the normal physiological scenario, dopaminergic cell bodies reside in the substantia nigra, from which they extend long axonal projections along the trajectory of the medial forebrain bundle (MFB) to the striatum where dopamine transmission is required. To date, ectopic placement of grafts in the striatum has been favored over homotopic, intra-nigral delivery. This is because intra-nigral transplantation is associated with poorer cell survival and insufficient striatal innervation, most likely caused by the absence of guidance cues along the already developed nigrostriatal pathway (Bentlage *et al.*, 1999) and the corresponding presence of inhibitory factors (Kauhausen *et al.*, 2015; Wictorin *et al.*, 1990). Early studies showed that

while dopaminergic neurons survive intra-nigral grafting, they failed to extend axons along the nigrostriatal pathway or produce any behavioral recovery (Bjorklund *et al.*, 1983b). These failings were understood to be due to the restrictive host environment and not a reflection of the cells capacity to grow. Proof-of-principle arose from the micro-transplantation of VM cells to the substantia nigra of 6-hydroxydopamine lesioned neonates, where nigrostriatal reconstruction was observed upon adulthood (Nikkhah *et al.*, 1995). Moreover, the transplantation of "bridge" grafts of Schwann cells that stretch from the transplantation site to the striatum alongside intra-nigral VM grafts showed that grafted dopamine neurons had the intrinsic potential to extend axons to the denervated striatum (Brecknell *et al.*, 1996; Wilby *et al.*, 1999). Xenografting studies have also shown that human VM cells placed into the substantia nigra of hemi-Parkinsonian adult rats could innervate regions of the host striatum, alluding to the concept that this was a result of the outgrowing axons' failure to recognise species-specific inhibitory factors (Isacson *et al.*, 1995). Additionally, recent studies using VM tissue from GFP transgenic mice have shown a notable pattern of axon growth towards the striatum, along with the normalisation of rotational behavior (Gaillard *et al.*, 2009; Kauhausen *et al.*, 2013; Thompson *et al.*, 2009).

Impact of neuronal subtype

Dopaminergic neuron subtype is another factor that holds influence over the efficacy of VM transplants. As they are dissected from primary tissue, VM grafts are heterogeneous with respect to cell type, with the dopaminergic neurons being just one component. Dopaminergic neurons can be further divided into three major cell groups, A8, A9 and A10, based on the classification of cerebral monoamine neurons by Dahlstrom and Fuxe (1964). A10 neurons are phenotypically small round cells that send projections to the cortical and limbic structures including the amygdala, nucleus accumbens, hippocampus and prefrontal cortex to form the mesocorticolimbic structure. A9 neurons are phenotypically larger angular cells that send projections predominantly to the dorsolateral striatum to form the nigrostriatal pathway. While, A8 neurons innervate limbic and striatal structures and provide local innervation to both A9 and A10 neurons (Bjorklund *et al.*, 2007). The A9 neurons are the most vulnerable to degeneration in Parkinson's disease, while the A10 neurons are relatively resistant to disease pathology and are one of the last to degenerate (Damier *et al.*, 1999). Moreover, the A9 component of VM grafts have been found to be the most important for functional recovery due to their exceptional ability to target the dorsolateral striatum which is involved in movement (Grealish *et al.*, 2010), highlighting the importance of a subtype ratio that is

favorable to A9 neurons. **Quantification of DA neuronal subtypes expressing A9 and A10 marker proteins** (G protein-gated inwardly rectifying potassium channel (Girk2) and calcium binding protein (calbindin), respectively) shows that intra-striatal VM grafts are comprised of 60-70% A9 neurons and 30-40% A10 neurons (Bye *et al.*, 2012). Interestingly, it was found that A9 neurons precede the birth of A10 neurons and as a result the use of younger embryonic donor tissue generated grafts which were composed of ~75% A9 neurons (Bye *et al.*, 2012). Furthermore, recent studies have demonstrated that A9 neurons from younger embryonic donor tissue are more responsive to environmental cues at the transplantation site when adopting a dopaminergic phenotype during differentiation post-grafting (Fjodorova *et al.*, 2017; Kauhausen *et al.*, 2013).

Impact of donor age

As highlighted above, embryonic donor age is of particular importance in VM transplantation. The VM must be collected during a specific time frame of neurogenesis to allow for the level of maturity required for commitment to a dopaminergic neuron phenotype, while also avoiding significant axonal outgrowth prior to tissue dissociation (as dissecting at this stage will sever the axons and kill the neurons). Early experiments established the upper limits of donor age for VM cell suspensions to be embryonic day (E) 15-16 (rat) (Brundin *et al.*, 1985) and in more recent years the conventional donor age has become E14 (rat) as it was perceived to coincide with peak dopamine neurogenesis (Hegarty *et al.*, 2013). More recently, the influence of donor age on graft efficacy has received heightened interest. Torres and colleagues investigated the yield of dopaminergic neurons from intra-striatal grafts of VM dissected at E11, E12, E13, E14 and E15, and found that the E12 preparations yielded the highest dopaminergic neurons at 5-fold that of the E14 preparation (Torres *et al.*, 2007). The authors hypothesised that the enhanced survival with this younger donor age may be a result of trophic support from the attached meningeal layer, a component that is ordinarily removed in E14 preparations. Moreover, a recent study showed that the addition of meningeal cells from young donors with VM preparations enhanced both the survival and axonal outgrowth of dopaminergic neurons (Somaa *et al.*, 2015).

Cellular brain repair for Parkinson's disease – Clinical studies

The positive results found in the early pre-clinical studies led to a swift movement to clinical trials, with the first open label clinical trial taking place less than 10 years after dopaminergic neurons from VM grafts were first shown to be suitable for transplantation. The first clinical

trials were carried out in the 1980s, firstly by Madrazo and colleagues in Mexico (Madrazo *et al.*, 1988) and then by Lindvall and colleagues in Sweden (Lindvall *et al.*, 1989). In both studies, 2 patients received an intra-striatal transplantation of human VM tissue from 12-14 and 8-10 week old embryos, respectively. While Madrazo and colleagues reported dramatic motor improvements, particularly in the disappearance of rigidity and dyskinesia, Lindvall and colleagues noted minimal clinical improvement. This lead to refinements in surgical technique such as a reduction in the diameter of implantation device and an increase in the number of implantation sites. As a result, in a subsequent study, Lindvall and colleagues reported a significant reduction in the rigidity and bradykinesia of two patients, with a marked decrease in the patients "on-off" phenomenon (Lindvall *et al.*, 1990). Moreover, long-term follow up showed that the grafted VM cells were capable of surviving and exerting functional benefit 3 years after transplantation (Lindvall *et al.*, 1994). This led to several subsequent open label trials which reported significant improvements in UPDRS scores, quality of life and levodopa requirements (Brundin *et al.*, 2000; Freed *et al.*, 1992; Hauser *et al.*, 1999; Peschanski *et al.*, 1994; Spencer *et al.*, 1992; Wenning *et al.*, 1997). Importantly, these positive results were associated with an increase in fluorodopa uptake, as measured by [^{18}F]-DOPA positron emission topography (PET) which is widely used as a measure of graft viability (Piccini *et al.*, 2005). Likewise, the binding of [^{11}C]-raclopride, a D_2 receptor antagonist, showed that the transplanted grafts restored D_2 receptor occupancy to normal levels. Encouragingly, the grafts' capacity to release dopamine can be maintained for at least a decade, despite their exposure to the ongoing disease progression (Piccini *et al.*, 1999). Furthermore, short-term (18 and 19 months) (Kordower *et al.*, 1998; Kordower *et al.*, 1995) and long-term (14 years) (Mendez *et al.*, 2008) *post mortem* analysis has shown that grafted dopaminergic neurons can survive the procurement and transplantation process, are capable of reinnervating the denervated striatum and can form graft-to-host synapses. Moreover, Kefalopoulou *et al*. (2014) recently reported on the long term (18 years after transplantation) symptomatic relief in two patients along with their discontinuation of any anti-Parkinsonian medication.

The success of the open-label trials led to the initiation of two double-blind, placebo-controlled trials in the early 2000s. The first, by Freed and colleagues (2001), included 40 patients between the age of 34 and 75 years with a mean disease duration of 14 years. They were randomly assigned to receive bilaterally either a fetal cell transplantation (tissue from two embryos/side, each between 7 and 8 weeks of age) or sham surgery. No

immunosuppression was given pre or post-operatively, and follow-up continued for 12 months post-transplantation. The primary outcome was a subjective self-report global rating of clinical improvement at 12 months post transplantation. Although [^{18}F]-DOPA PET and *post mortem* analysis confirmed the survival and growth of transplanted grafts, the study failed to reach its primary endpoint, with no significant difference found in total UPDRS scores between the treatment and placebo groups. However, significant improvement in "off" state UPDRS and motor scores were found in transplant patients below 60 years of age. Of concern, 5 of 33 patients who received a transplant (including those from the sham group who elected to have the surgery after the study) developed dyskinesias within the first year. These dyskinesias persisted after the reduction or cessation of dopaminergic medication and are now known as graft-induced dyskinesias (GIDs). Subsequent pre-clinical, as well as retrospective clinical assessments of patient transplants, indicated that the presence of serotonergic neurons within the donor preparations (a consequence of poor/broad tissue dissection and isolation of hindbrain nuclei), as well as uneven striatal reinnervation by the grafts were likely responsible for the observed GID (Carlsson *et al.*, 2007; Carlsson *et al.*, 2006; Hagell *et al.*, 2002). The second placebo-controlled trial included 34 patients between the ages of 30 and 75 years of age (Olanow *et al.*, 2003). Patients were randomly assigned to receive bilaterally either a fetal cell transplantation (from one or four embryos/side, each between 6 to 9 weeks) or sham surgery. All patients received immunosuppression with cyclosporine 2 weeks pre-operatively and up to 6 months post-operatively. The primary outcome measure was a significant difference in the "off" state UPDRS score from baseline to the final 24-month visit. Again, the primary endpoint was not achieved, although there was a trend towards improved motor scores in patients who received a 4 embryo transplant. Stratification based on disease severity, also showed significant improvement in motor scores in less severe patients who received 4 embryo transplants. Patients also showed significant motor improvement at 6 and 9 months post-transplant with deterioration afterwards, which may be a result of the cessation of immunosuppression. *Post mortem* analysis did show good survival of dopaminergic neurons and re-innervation of the striatum, while PET analysis revealed increases in fluorodopa uptake. However, worryingly, within a year GIDs were found in several grafted patients, similar to the previous findings of Freed and Colleagues (Freed *et al.*, 2001). **Further to this, numerous *post mortem* reports (>10 years post-transplant) have shown that a number of dopaminergic neurons grafted into the putamen of Parkinson's disease patients display Lewy body pathology that is indistinguishable from those seen in the host brain (Chu *et al.*, 2010; Kordower *et al.*,**

2008a; Kordower *et al.*, 2008b; Li *et al.*, 2008; Li *et al.*, 2010), highlighting that disease progression continues in the face of transplantation, a process that could have detrimental effects to the long term efficacy of grafted cells.

The results of these trials raised concerns over the efficacy and safety of fetal VM transplants in Parkinson's disease, and while the reasons behind the negative outcomes and GIDs remain unknown, patient section, tissue preparation, tissue placement, immunosuppression and follow-up time are all thought to be contributing factors. The best results from these placebo-controlled trials were found in patients with lower disease severity (rated by UPDRS scores) and good levodopa response pre-transplantation, suggesting that disease severity is a factor that could affect the efficacy of transplantation. Additionally, large variations in tissue preparations were seen between the studies. Freed *et al*. (2001) delivered a lower volume of tissue that had been stored for 4 weeks prior to transplant, while Olanow *et al*. (2003) delivered larger quantities of tissue that was only stored for 2 days prior to transplant. The long storage of tissue prior to transplant may have been deleterious to cell survival, which could be further affected by the lower quantity of tissue delivered. Further to this, donor age (range of 6-9 weeks) and tissue composition (strands vs. pieces) **are other factors that could have affected graft efficacy**. Freed *et al*. (2001) also implemented a new trajectory to the striatum which may have affected cell distribution. The breach of the blood-brain-barrier (BBB) during surgery and the subsequent addition of a cell transplant are both factors that can instigate a host immune response, therefore the decision to implement (Olanow *et al*., 2003) or not (Freed *et al*., 2003) an immunosuppressive regimen may have affected graft survival. The decision to stop immune suppression after 6 months in the Olanow *et al.* (2003) study is notable because up until this point, grafted patients had improved at a rate similar to that seen in the open label studies. The deterioration after cessation of immunosuppression could be explained by a delayed immune response that compromised long-term survival. Concern also rose over the decision to base the primary outcome on results found at just one year. Open label studies had shown that graft-induced recovery can take months to years to develop. A follow-up of the Olanow *et al*. (2003) study at 2 and 4 years post-transplant showed significant improvements in UPDRS motor scores and flurodopa uptake. Moreover, the increase in flurodopa uptake over the course of the study correlates with the clinical outcome (Ma *et al.*, 2010).

Cellular brain repair for Parkinson's disease – Potential of biomaterials

One of the major issues that has limited the efficacy of fetal VM grafts since the initial pioneering studies began ~40 years ago, is the poor survival and engraftment of the dopaminergic neurons after transplantation in to the brain. The clinical and pre-clinical experiences gained so far have all reported poor survival of dopaminergic neurons after transplantation, estimated at less than 20%, see reviews - Castilho et al., (2000); Olanow et al., (1996). This extremely poor survival results in the need for multiple fetal donors per transplant, thus further exacerbating the ethical concerns over the use of tissue from elective abortions.

It was previously assumed that cell death in VM grafts was predominantly necrotic occurring as a result of cell insult during the tissue dissection and transplantation process. However, while some necrotic cell death does occur, the large extent of cell death in VM grafts occurs post-transplantation through apoptosis and is predominantly driven by external factors in the cells' environment rather than a physiologic insult (Castilho *et al.*, 2000; Mahalik *et al.*, 1994; Schierle *et al.*, 1999; Sortwell *et al.*, 2000; Zawada *et al.*, 2001). Apoptotic cell death is triggered at various points of the transplantation process by factors such as 1) detachment from the extracellular matrix during tissue dissection (Reddig *et al.*, 2005), 2) shearing of the cells upon delivery, via fine cannulas, into the host parenchyma (Barker *et al.*, 1995), 3) immediate growth factor deprivation upon transplantation into the adult striatum (Collier *et al.*, 1999), and 4) the recruitment of host neuro-immune cells to the graft (Duan *et al.*, 1995). Each of these stages provides an intervention point at which graft survival could be improved. Consequently, a number of studies explored the benefit of anti-apoptotic agents such as caspase inhibitors/lazaroids and JNK inhibitors (Karlsson *et al.*, 2002; Rawal *et al.*, 2007; Schierle *et al.*, 1999), **as well as pro-survival proteins inclusive of glial-derived neurotrophic factor (GDNF) and Neurturin, which have shown to be advantageous to dopaminergic neuron survival, at the stages of cell preparation, implantation and/or integration – see review Deierborg *et al.* (2008).** More recently however, data has begun to emerge which shows that injectable biomaterial scaffolds, such as *in situ* forming hydrogels, have the potential to improve the engraftment and survival of these grafts in the brain through provision of a supportive microenvironment for cell adhesion, growth and protection from the host immune response. Thus, since cell death in primary dopaminergic cell grafts occurs over a number of distinct stages throughout the whole transplantation process, for a biomaterial scaffold to be advantageous to the delivery, survival and efficacy of cell replacement efforts it should be capable of 1) providing a supportive environment for cell adhesion, 2) providing

a reservoir for localised and sustained growth factor delivery, and 3) creating a physical barrier between the transplanted cells and the host neuro-immune cells (Fig. 1).

Cell-matrix adhesion

Prior to transplantation, embryonic tissue must be collected, dissected and dissociated into a cell suspension. Even with the clean and efficient dissection of VM tissue, mechanical destruction caused by cellular detachment from the extracellular matrix during dissociation removes the normal cell-matrix interactions and cell death can ensue (Marchionini *et al.*, 2003; Reddig *et al.*, 2005). This process, known as anoikis, was first termed by Frisch and Francis when they showed that the loss of integral cell-matrix interactions is a major trigger of apoptotic cell death (Frisch *et al.*, 1994). Hence, the delivery of cells in a biomaterial matrix to which they can adhere may provide them with the necessary support needed both during and after transplantation. **Indeed, studies have shown that the attachment of neural cells to biomaterials scaffolds (particularly compressive biosynthetic materials) prior to transplantation can provide cells with an adherent surface throughout the transplantation process, therefore reducing the detrimental effects of anoikis on cell survival (Beduer *et al.*, 2015; Jgamadze *et al.*, 2012; Moriarty *et al.*, 2018a&b; Moriarty *et al.*, 2017; Newland *et al.*, 2015).**

Growth factor provision

Fetal cell replacement therapy in Parkinson's disease involves the removal of dopaminergic neurons from the VM of the developing embryo and subsequent transplantation into the adult striatum. As the trophic activity of the brain and in particular, the striatum, is known to decrease with age (Ling *et al.*, 2000), these cells are removed from a trophic-rich environment at the height of neurogenesis and placed into the depleted striatum. As a result, transplanted cells undergo trophic withdrawal, being deprived of the factors normally present throughout target innervation and development (Abeliovich *et al.*, 2007). Indeed, numerous studies suggest that the critical time-point in which 80-90% of dopaminergic neurons die is the first 4 days post-transplantation, and that it is not until after this point that dopaminergic neuron survival is stabilised (Barker *et al.*, 1996; Emgard *et al.*, 1999; Rawal *et al.*, 2007; Sortwell *et al.*, 2001; Sortwell *et al.*, 2000). **The incorporation of growth factors, such as brain-derived growth factor (BDNF), Neurotrophin-3 (NT-3), Neurotrophin-4/5 (NT-4/5), growth/differentiation factor 5 (GDF5) and GDNF, have been investigated for their potential to improve dopaminergic cell transplantation strategies (Clayton *et al.*, 2007;**

Feng *et al.*, 1999; Hyman *et al.*, 1994; Jaumotte *et al.*, 2014; Lin *et al.*, 1993). While they have all shown to improve dopamine neuron survival in VM cultures, GDNF has been established as an extremely potent neurotrophic factor, significantly improving cell survival and efficacy (Apostolides *et al.*, 1998; Chaturvedi *et al.*, 2003; Deng *et al.*, 2013; Redmond *et al.*, 2013; Rosenblad *et al.*, 1996; Yurek *et al.*, 2009). However, the encapsulation of cells in a growth factor-loaded biomaterial matrix has the potential to further enhance cell survival and efficacy by providing the transplanted cells with localised, site-specific and prolonged access to growth factors upon transplantation and the period of target innervation.

Hydrogels from a variety of sources, both natural and synthetic, have shown to successfully deliver trophic factors to the brain in a site-specific, controlled and sustained manner (Chierchia *et al*., 2017; Fon *et al*., 2014; Li *et al*., 2016). Further to the enhanced delivery of GDNF in injectable hydrogels (Fon *et al*., 2014), many approaches have investigated the use of hollow micro-particles to achieve sustained GDNF release from a single administration (Agbay *et al*., 2014; Garbayo *et al*., 2016; García-Caballero *et al*., 2017; Lampe *et al*., 2011). Moreover, the delivery of GDNF containing microspheres in an injectable fibrin hydrogel enhanced the length of GDNF release *in situ* to 2 weeks compared to 3 days with "free" GDNF (Wood *et al*., 2013). Similarly, hydrogels have also been successfully used to enhance cellular delivery (Aguado *et al*., 2012; Ballios *et al*., 2015; Das *et al*., 2016; Freudenberg *et al*., 2009). Three recent studies have highlighted the potential of injectable hydrogels to improve dopaminergic cell replacement strategies. First, in the study by Wang and colleagues**, a GDNF-functionalised composite poly(l-lactic acid)/xyloglucan hydrogel, where GDNF was blended into and/or covalently attached to the scaffold,** was shown to enhance survival of, and striatal re-innervation from, transplanted mouse VM grafts in Parkinsonian mice (Wang *et al.*, 2016). Second, Adil and colleagues showed that a heparin/RGD functionalised hyaluronic acid hydrogel could improve the survival of transplanted human embryonic stem cell-derived dopaminergic neurons (Adil *et al.*, 2017). Finally, Moriarty *et al*. (2017, 2018b) demonstrated that encapsulating VM grafts in a GDNF-**loaded** collagen hydrogel resulted in a dramatic increase in the survival of dopaminergic neurons and that this correlated with enhanced striatal re-innervation and restoration of motor function in hemi-Parkinsonian rats.

Immune shielding

Experimental studies have shown that the injection of exogenous cells into the brain evokes an elevated host immune response (Barker *et al.*, 1996; Duan *et al.*, 1997; Duan *et al.*, 1993; Hudson *et al.*, 1994; Shinoda *et al.*, 1995). Additionally, in line with the prominent cell death seen immediately post-transplantation, microglial activation, lymphocyte infiltration and major histocompatibility complex (MHC) expression all increased over the first 4 days post-transplantation (Duan *et al.*, 1995). Moreover, the immune response may intensify over-time as the immune cells in the brain are not stationary and inactive, but rather provide a continuous inflammatory response (Shinoda *et al.*, 1995). *Post mortem* analysis from the double-blind, placebo-controlled clinical trials, as well as an earlier study, detected prominent activated microglial staining around the graft site (Freed *et al.*, 2001; Kordower *et al.*, 1997; Olanow *et al.*, 2003). This immune response against the transplanted cells may have hindered their survival and consequently had an effect on their clinical efficacy. This seems increasingly possible in the Olanow *et al*. trial where deterioration of clinical benefit began after the withdrawal of immunosuppression (Olanow *et al.*, 2003). Thus, encapsulation of cells within a supportive biomaterial matrix may protect the transplanted cells from the hostile host environment by forming a physical barrier between the transplanted cells and the host neuro-immune cells. Indeed, Hoban *et al*. (2013) and Moriarty *et al*. (2017) have both demonstrated a dramatic reduction in the recruitment and proliferation of microglia and astrocytes around intrastriatal cell grafts when encapsulated in a collagen hydrogel.

Cellular brain repair for Parkinson's disease – Which biomaterial to use?

Repairing the damaged brain can be a daunting task, yet recent advances in tissue engineering and cell-based therapies are bringing us closer to clinical translation. The diversity and adaptability of biomaterial scaffolds makes them an attractive strategy for neural cell replacement therapy (Orive *et al.*, 2009), however, any material used for intra-cranial delivery should exhibit a number of desirable characteristics. Such materials should 1) be capable of relatively non-invasive delivery 2) be biomimetic in order to encourage cell survival and host integration, 3) not themselves elicit an exaggerated host immune reaction that can instigate neuroinflammation around the transplantation site, 4) be structurally stable for prolonged periods *in situ* and where appropriate biodegrade without leaving any undesirable foreign remnants, 5) be modifiable in relation to adhesion molecules, pore size, molecular charge, surface topography and functionalisation, 5) be non-toxic to any cellular components of brain tissue or the encapsulated cells and 6) be capable of controlled and

sustained delivery of therapeutic factors (based on Orive *et al.*, (2009) and Wang et al., (2012)).

Injectable hydrogels as a biomaterial scaffold

The large range of available biomaterials coupled with their high adaptability leads to the generation of application specific materials, making biomaterials a very attractive avenue in the field of cell-based therapies (Kim *et al.*, 2012). Biomaterials can be characterised under two main subtypes, natural materials or synthetic materials. Natural materials are derived from biological sources including, chitosan, alginate, methylcellulose, hyaluronan, fibrin and collagen. The advantages of their use stems from their natural roles in the biological system. Many contain endogenous binding sites that allow for natural cell adhesion (Heino *et al.*, 2009), while their biological source minimises the activation of the host immune response (Mano *et al.*, 2007). In comparison, synthetic materials are chemically manufactured and can therefore be more readily manipulated and standardised (Lutolf *et al.*, 2005). **Indeed, many biomaterials that use natural materials, such as alginate, fibrin or collagen, as their primary framework are often crosslinked with synthetic polymers, such as polymer polyethylene glycol (PEG), giving rise to a new class of biosynthetic materials which possess the biological properties of the protein and the mechanical stability afforded by the chemical crosslinking (Delgado *et al.*, 2015)**. It is of upmost importance when choosing a biomaterial, whether it be from a natural, synthetic or **biosynthetic** origin, to take their individual characteristics into consideration, as properties such as adhesion potential, degradability, shape, pore size, hydrophilicity and delivery potential will render them suitable or unsuitable for specific applications.

In relation to the CNS, injectable hydrogels are the most widely investigated and promising biomaterial scaffolds for the delivery of therapeutic agents and/or cells to the brain in regenerative therapies (Burdick *et al.*, 2016). Hydrogels are three-dimensional networks of hydrophilic polymers which can be chemically crosslinked to form insoluble polymer matrices (Hoffman, 2002). The ability of hydrogels to form *in situ* in response to temperature and pH changes makes them injectable, an extremely attractive property which allows for their relatively non-invasive intra-cranial delivery (Pakulska *et al.*, 2012). Furthermore, hydrogels can be chemically crosslinked to alter their physical properties to specific applications. Indeed, the degree of chemical crosslinking used can directly affect the level of gelation (strength of *in situ* formation), porous structure and degradation (Drury *et al.*, 2003).

The alteration of a hydrogel's porous structure allows for control over nutrient infusion to encapsulated cells and therapeutic factor diffusion to surrounding tissues (Lee *et al.*, 2016), while simultaneously minimising host immune cell infiltration. Additionally, by using biomaterials that undergo natural degradation, they will eventually be eliminated from the body, while the degree of chemical crosslinking used can control the hydrogels degradation rate and therefore its persistence *in situ (*Davidenko *et al., 2015)*. Depending on their biological source, hydrogels may naturally support cell adhesion or be manipulated to support cell attachment through the addition of adhesion factors (Hersel *et al.*, 2003). Moreover, growth factors can be added to further support cell survival and function after transplantation (Burdick *et al.*, 2016) with studies now demonstrating the capacity to temporally control the release of multiple growth factors simultaneously or sequentially, dependent on the requirement of the host and/or inplanted cells (Bruggeman *et al.*, 2016).

Injectable collagen hydrogels

Forming 20-30% of the body's protein component, the collagen family is the body's most abundant protein group, making collagen one of the most investigated natural biomaterials (Khan *et al.*, 2013). There are many distinct types of collagen found throughout the body, however, 90% of collagen in the human body is type I (Henriksen *et al.*, 2016). Type 1 collagen can be extracted with ease from animal tissues including tendons and skin. It is a fibrous protein composed of three polypeptide chains (α subunits) that are wound together using hydrogen bonds to form a triple helix structure (Bhattacharjee *et al.*, 2005). The transition of collagen from a liquid to a solid state through the structural change of the triple helix to highly compacted coils as a result of physiological conditions such as temperature and pH, makes it an attractive biomaterial for CNS delivery (Sargeant *et al.*, 2012). Furthermore, this *in situ* gelation makes collagen hydrogels an ideal delivery scaffold for both trophic and cellular regenerative therapies.

While collagen is capable of naturally forming a hydrogel *in situ*, its weak mechanical properties make it highly susceptible to rapid degradation in the brain. In an effort to better control the mechanical stability of collagen, the use of synthetic polymers to form biosynthetic hydrogels that possess the biological properties of the protein and the mechanical stability afforded by the chemical crosslinking has been investigated (Delgado *et al.*, 2015; Sargeant *et al.*, 2012). The synthetic polymer PEG is hydrophilic, non-toxic, non-immunogenic (Veronese *et al.*, 2005) and importantly, already FDA approved for a number

of clinical applications (Alconcel *et al.*, 2011). Furthermore, the chemical cross-linking of collagen with PEG has shown to improve the mechanical stability, degradation rate and protein diffusion from these biosynthetic hydrogels, while also maintaining the proteins biological function (Doillon *et al.*, 1994; Lee *et al.*, 2000; Sargeant *et al.*, 2012; Weber *et al.*, 2009). Thus, the ability of cross-linked collagen hydrogels to mimic the extracellular matrix, while also having control over the strength of gelation, rate of degradation and diffusion of encapsulated factors makes them an extremely attractive biomaterial scaffold for cell replacement therapies.

Keeping with this, collagen biomaterials have already been approved for use in a variety of applications including drug delivery, wound repair, burn treatment, dentistry and bone reconstruction (Blume *et al.*, 2011; Chajra *et al.*, 2008; Chattopadhyay *et al.*, 2014; El-Chaar, 2016; Helary *et al.*, 2010; Khan *et al.*, 2013; Parenteau-Bareil *et al.*, 2010; Patino *et al.*, 2002; Solish, 2010) and could therefore be relatively easily adopted to neural applications. While the intra-cranial use of collagen hydrogels has been looked at to a lesser extent, Hoban *et al*. (2013) and Moriarty *et al*. (2017, 2018b) have recently demonstrated the efficacy of PEG crosslinked collagen hydrogels for brain repair in Parkinson's disease. Thus, given the clinically biocompatible, immunoprotective and supportive properties of collagen hydrogels, they hold immense potential to improve the survival and efficacy of dopaminergic cell replacement therapies in Parkinson's disease. Taken together, this literature highlights the potential of biomaterial hydrogel scaffolds to improve the outcome of reparative cell therapies for Parkinson's disease. Moving towards a clinical therapy, the use of biomaterial scaffolds from natural polymers, namely collagen, offers significant translatability owing to it already being approved for use in a variety of clinical applications (Chajra *et al.*, 2008; Chattopadhyay *et al.*, 2014; Patino *et al.*, 2002; Solish, 2010).

Conclusion

As cell therapies for Parkinson's disease and other neurodegenerative disorders propel towards the clinic, it is of increasing importance to address strategies to ensure maximal survival, integration and functional efficacy of the newly implanted tissue. In this regard, it is clear that evidence is mounting that supports the potential of biomaterial scaffolds to enhance brain repair for Parkinson's disease. Further work remains to be carried out to identify the ideal biomimetic scaffold, and determine optimal strategies to functionalise these matricies – targeted at supporting cell transplant for neural repair.

Conflicts of interest:

The authors declare no competing financial interests**.**

Contributions:

We confirm that this manuscript has been read and approved by all named authors and that all named authors have fulfilled the required criteria for authorship. N.M, C.P and E.D contributed equally to the drafted paper.

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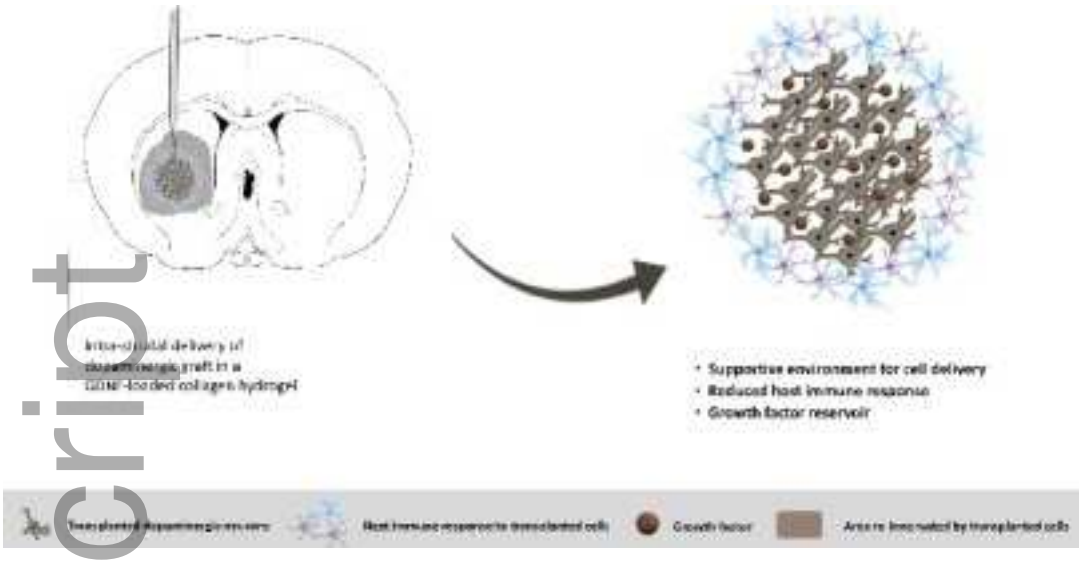
Figure legend

Fig. 1. Therapeutic concept of biomaterials for brain repair in Parkinson's disease. Encapsulation of transplanted dopaminergic neurons in a glial-derived neurotrophic factor (GDNF)-functionalised collagen hydrogel could improve brain repair in Parkinson's disease through a number of different mechanisms. These include provision of 1) a physical scaffold for cell adhesion during intracerebral delivery and engraftment, 2) a local reservoir for GDNF at the implantation site, and 3) a protective barrier against the host immune response. Taken from Moriarty *et al*. (2018a).



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