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Harnessing stem cells and biomaterials to promote neural repair

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Abbreviations: bFGF, basic fibroblast growth factor; BDNF, brain derived neurotrophic factor; DA, dopamine; ESC, embryonic stem cells; ECM, extracellular matrix; GDNF, glial cell line derived neurotrophic factor; IPSC, embryonic stem cells; NGF, nerve growth factor; PD, Parkinson's Disease; PSC, pluripotent stem cells; SAP, self-assembling peptides; TEM, Transmission electron microscopy; VM, ventral midbrain

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Abstract

With the limited capacity for self-repair in the adult central nervous system, efforts to stimulate quiescent stem cell populations within discrete brain regions, as well as harness the potential of stem cell transplants, offers significant hope for neural repair. These new cells are capable of providing trophic cues to support residual host populations and/or replace those cells lost to the primary insult. However, issues with low level adult neurogenesis, cell survival, directed differentiation, and inadequate reinnervation of host tissue have impeded the full potential of these therapeutic approaches and their clinical advancement. Biomaterials offer novel approaches to stimulate endogenous neurogenesis, as well as for the delivery and support of neural progenitor transplants, providing a tissue-appropriate physical and trophic milieu for the newly integrating cells.

In this review we will discuss the various approaches by which bioengineered scaffolds may improve stem cell-based therapies for repair of the central nervous system.

Introduction

With an increasingly ageing population the incidence of chronic neurodegenerative disorders, such as Parkinson's disease, Huntington's disease, Alzheimer's disease and amyotrophic lateral sclerosis, as well as acute insults including ischemic and haemorrhagic stroke are on the rise. Each of these are characterised by cognitive, sensory and/or motor impairments underpinned by loss of neuronal subpopulations (Lindvall & Kokaia, 2010). In both instances, chronic and acute injuries, approaches are needed to manage the neurological deficits induced by this cell loss. However, current therapeutic options predominantly focus on managing symptoms using drugs, physical therapy, and deep brain stimulation (Lindvall & Kokaia, 2010). Consequently, the development of new and novel therapies is in critical demand.

Unlike many tissues, the central nervous system has limited capacity for repair. Until the 1960s it was thought that we were born with our complete complement of neurons, since then discrete pockets of new neurons have been identified in the adult brain (Altman, 1962). These new cells, however, are seemingly few in numbers and, despite increased numbers following injury (within these discrete locations in the brain) are unable to restore neuronal numbers lost to disease or injury. Persistent research strives to understand the mechanisms that underpin adult neurogenesis, with the hope of exploiting these processes to enhance repair (Lindvall & Kokaia, 2011; Lindvall & Kokaia, 2015).

Recognising the limited capacity for self-repair in the adult brain, an alternate experimental approach has been the replacement of lost neurons through transplantation. Most effectively demonstrated using embryonic tissue, pre-clinical and clinical trials have provided proof of principle that newly implanted neurons can survive, structurally integrate, and alleviate disease-associated symptoms (Kirkeby, Parmar & Barker, 2017). Since the discovery of pluripotent stem cells, and the significant advancement in the directed differentiation of these cells into restricted neuronal fates, the feasibility of cell replacement therapies is becoming increasingly recognised (Barker, Parmar, Studer & Takahashi, 2017; Kokaia & Lindvall, 2012).

Despite the therapeutic potential of these two approaches targeted at brain repair (i.e. stimulation of endogenous neurogenesis and cell transplantation), both present ongoing challenges for the field including: adequate cell numbers/tissue availability, survival, directed differentiation, and appropriate integration into the host tissue (Barker, Parmar, Studer & Takahashi, 2017; Castilho, Hansson & Brundin, 2000; Kokaia & Lindvall, 2012; Lindvall & Kokaia, 2010). Research has shown that exposure of stem cells to the appropriate physical and chemical environment improves fate specification and cellular integration, however, it can be difficult to provide these cues in temporally and spatially appropriate manners *in vivo* (Kirkeby et al., 2012; Kriks et al., 2011; Niclis et al., 2017a; Shi, Kirwan, Smith, Robinson & Livesey, 2012).

In an effort to improve cell-based therapies for neural repair, bioengineered scaffolds have been proposed. Biomaterials are being investigated for their potential to restore tissue architecture, enhance cell survival and differentiation, as well as promote plasticity and integration of both endogenous and transplanted stem cells (Jendelova, Kubinova, Sandvig, Erceg, Sandvig & Sykova, 2016; Moriarty, Parish & Dowd, 2018; Rodriguez, Nisbet & Parish, 2012). In this review, we will discuss the progress (and challenges) in stimulating endogenous repair and cell transplantation from the perspective of 2 common neural injuries; one that is chronic yet discrete in nature (Parkinson's disease), and an acute injury that is notably more diffuse and complex in its requirements for repair

(stroke). We will describe the structural and functional benefits bioengineered scaffolds may have in advancing cell-based therapies for these, and other, neural injuries.

Cell Replacement Therapy for Parkinson's Disease

Parkinson's Disease (PD) is a movement disorder caused by progressive death of ventral midbrain (VM) dopamine neurons and subsequent degeneration of the nigrostriatal pathway (Winkler, Kirik & Bjorklund, 2005). Pharmacotherapy, using dopamine modulating drugs, is capable of restoring motor functions, yet has no effect on disease progression, is limited by side effects, and shows waning efficacy over time. Yet it was this evidence, restoring motor deficits with dopamine, that inspired cell replacement therapy for the disease. Dopamine progenitors, isolated from the developing fetal VM, were shown to survive, achieve synaptic integration, and restore dopamine transmission following implantation into Parkinsonian rodents and non-human primates, Figure 1 (Kirkeby, Parmar & Barker, 2017; Thompson & Parish, 2013). These findings led to a series of clinical trials through the 1990s that similarly demonstrated the capacity for these newly implanted dopamine progenitors to restore deficits for up to 24 years, despite ongoing disease progression (Winkler, Kirik & Bjorklund, 2005). However, the outcomes were highly variable – largely attributed to the source of the donor tissue. Fetal tissue, procured under consent from elective abortions was unreliable (Winkler, Kirik & Bjorklund, 2005). Considerable variability in donor tissue age, tissue integrity, dissection procedures, and microbiological status of the isolated tissue rendered it incompatible with the standardisation, quality control, and safety measures necessary for clinical application. Added to this were the ethical and bioavailability constraints of fetal tissue. Combined, this highlighted the need for an alternative, standardised donor source.

The discovery of human embryonic stem cells (ESC) in 1998 (Thomson, 1998), and subsequently of induced pluripotent stem cells (iPSCs) in 2007 (Takahashi et al., 2007), proclaimed new beginnings in regenerative medicine. Human ESC, derived from the inner cell mass of blastocysts procured from excess IVF embryos, and iPSCs, generated by genetic reprogramming of somatic cells, provide a sustainable cell source with the capacity to differentiate into restricted lineages – thereby providing an attractive cell source for cell-based therapies. Surprisingly, despite monumental efforts, only in recent years have protocols emerged that generate *bona fide* VM DA neurons (Kirkeby et al., 2012; Kriks et al., 2011). The key discovery that underpinned this advancement was the recognition that VM DA neurons originated from floorplate progenitor pools (and not the neuroectoderm, as once thought). Consequently, early ventralisation of the pluripotent stem cells in culture (via modulation of sonic hedgehog signalling) results in appropriate patterning and generation of developmentally relevant cells that progressively expressed markers such as FOXA2 and LMX1A (early DA progenitors), PITX3 (late DA progenitors), NURR1 (post-mitotic DA precursors), and tyrosine hydroxylase, TH (differentiated DA neuron) (Kirkeby et al., 2012; Kriks et al., 2011). Similar to fetal tissue studies, these PSC-derived DA progenitors are capable of surviving and functionally integrating into PD models, with the anticipation of clinical trials commencing by late 2018 (Barker, Parmar, Studer & Takahashi, 2017).

While *in vitro* differentiation routinely shows high proportions of correctly specified VM progenitors, at the time amenable to transplantation, the ability to predict their capacity to give rise to grafts rich in DA neurons remains a black box. Most evidently demonstrated by the group of Malin Pamar (Kirkeby et al., 2017), >30 independent human PSC differentiations of seemingly similar VM progenitor fate prior to grafting (>80% LMX1A+FOXA2+OTX2+) showed vastly different outcomes *in*

vivo – with some grafts showing no, or few TH+ dopamine neurons, whilst others contained high DA yields, capable of functional impacts. Such outcomes suggest that greater understanding and control of the differentiation of human PSCs remains to be achieved.

Added to this, and similarly observed in pre-clinical and clinical fetal tissue grafts, is the notably low proportion of DA neurons within the grafts, with most studies reporting between 3-8% of the total graft (Doi et al., 2014; Kirkeby et al., 2012; Kirkeby, Parmar & Barker, 2017; Kriks et al., 2011; Niclis et al., 2017b; Samata et al., 2016). Whilst functional assessments have revealed sufficient DA neurons for restoration of motor functions in rodent and non-human primate models, questions remain regarding the impact of the non-dopaminergic cells to graft function. Only recently, using reporter stem cell lines to specifically track the contribution of dopamine neurons to the graft, has the level of non-DA influence been recognised. Whilst assessment of young grafts (<6 weeks after implantation) reveals high proportions of DA progenitors, reflective on their *in vitro* identity, at more protracted time periods (>6 months), TH+ DA neurons only contribute to a fraction of the graft, suggesting failure of progenitor maturation and/or expansion of the incorrectly specified (yet remaining FOXA2+) cells from culture (Niclis et al., 2017b).

Obviously, the survival of DA neurons is of critical importance, being the most decisive factor in governing whether a transplant is successful in ameliorating symptoms yet is challenging at many levels. There are a number of key events that impact on their survival including: (I) - handling of the donor cells prior to implantation (inclusive of their dissociation, preparation medias, and storage); (II) - the physical process of implantation (noting the shear forces exerted on the cells during injection); and (III) - integration into the host tissue, recognising that the adult brain into which transplants are placed lacks many neurotrophic cues that are normally present when DA progenitors/neurons and their axons navigate their way *in situ* during development Studies report DA cell survival rates post implantation within the adult brain of <20% for fetal tissue grafts compared to <10% for human PSC-derived DA progenitor grafts (Castilho, Hansson & Brundin, 2000; Niclis et al., 2017b).

A further challenge for the field has been graft integration, with numerous studies observing that human PSC-derived DA transplants show notably poorer striatal innervation than their human VM fetal counterparts, with a recent direct comparative study reporting a 4-fold reduction within the target dorsolateral striatum (Grealish et al., 2014). Whilst a variety of trophic cues, namely glial cell line derived neurotrophic factor (GDNF), have been demonstrated to improve the survival and plasticity of fetal tissue grafts, their potential for PSC-derived grafts are only just beginning to be realised (unpublished data/under review). Moving forwards, in addition to the identification of trophic cues capable of supporting survival and plasticity of human PSC-derived grafts, will be the need for safe and efficient delivery approaches that are both spatially and temporally relevant to the duration of graft integration. While the past 40 years of research have made significant advancements in stem cell differentiation and transplantation for PD, a new wave of interest into the potential of bioengineered scaffolds to overcome some of the existing/persistent challenges has begun.

Cell-based therapy for stroke

Stroke, caused by disruption of blood flow to the brain, results in mortally injured necrotic core (primary injury) that is surrounded by the penumbra – vulnerable tissue that can also die (i.e. the secondary injury), but is potentially salvageable (Lindvall & Kokaia, 2011). With the exception of

thrombolytic drugs and surgical clot removal, both targeted at restoring blood flow and protection of the penumbra, no treatments are currently available to improve functional recovery in patients. In this regard, cell transplantation, to replace and/or protect circuitry, offers new and realistic hope (Lindvall & Kokaia, 2011).

However, unlike Parkinson's disease, the challenges of cell-based repair following stroke are notably greater. Here, the injury can affect many brain areas and involve a variety of cell populations. Furthermore, the task is notably greater than replacing a single discrete cell population, requiring all or many of the following: (i) restoration of gross tissue architecture; (ii) deployment of replacement cells; (iii) establishment of a suitable microenvironment for graft cells survival and integration, whilst also supporting endogenous cells in the penumbra; and (iv) ensuring vascularisation of the new tissue.

Evidently, a key hurdle for cell transplantation targeted at stroke is how best to get the implanted cells to survive and 'knit' into the host tissue when implanted into the necrotic core. The alternate is to transplant the cells into the penumbra/peri-infarct tissue, however this may risk damaging vulnerable and potentially salvageable tissue. Despite this, studies have demonstrated with varying success the capacity of adult or fetal-derived neural progenitors to survive, differentiate and functionally integrate into the stroke injured brain. These studies have also demonstrated the capacity of the grafted stem cells to migrate into the injury site and show electrophysical properties suggestive of mature neurons, figure 1 (Bacigaluppi, 2008; Lindvall & Kokaia, 2010).

Much like the limitations of fetal (and adult) NSC sources in PD, the availability of pluripotent stem cell lines has enabled a new wave of research into cell-based therapies for repair of the acutely injured CNS. With relatively little effort, human iPSCs and ESCs can be differentiated with high efficiency into neural progenitors, by dual Smad inhibition (inhibition of TGFβ and BMP pathways) (Chambers, Fasano, Papapetrou, Tomishima, Sadelain & Studer, 2009; Shi, Kirwan, Smith, Robinson & Livesey, 2012). These PAX6+ dorsal forebrain progenitors present a neural population that conveniently aligns with the common stroke models that induce infarcts within the cortex. Following transplantation, these PSC-derived neurons exhibit a remarkable capacity for long-distance anatomical integration and establishment of appropriate electrophysiological properties (Denham et al., 2012; Espuny-Camacho et al., 2013; Niclis et al., 2017c) and, similar to fetal and adult NSC grafts, provide varying degrees of functional recovery (Chan-Ling, Daadi, Maag & Steinberg, 2008; Gomi et al., 2012; Jensen, Yan, Krishnaney-Davison, Al Sawaf & Zhang, 2013; Jiang et al., 2011; Oki, 2012; Qin et al., 2013; Tatarishvili, 2014; Tornero et al., 2013). Behavioural recovery, however, has commonly been observed over timeframes inconsistent with the maturity of implanted human neurons, thereby making it difficult to assess the direct contribution of these cells to neuronal replacement.

Research groups have also explored the capacity of immortalised neural stem cell lines, as well as non-neural stem cell sources (inclusive of olfactory ensheathing cells) to promote repair (Jendelova, Kubinova, Sandvig, Erceg, Sandvig & Sykova, 2016; Lindvall & Kokaia, 2011). These alternate stem cell sources have the advantage of accessibility and availability. While some degree of neuronal differentiation and functional integration into host neural circuitry has been reported, the general consensus from studies transplanting these immortalized NSC lines and non-neural stem cells into the stroke injured brain (and similarly in traumatic brain and spinal cord injuries) is that the implanted cells act in a paracrine manner – promoting varying degrees of functional recovery through mechanisms other than cell replacement – see reviews (Jendelova, Kubinova, Sandvig, Erceg, Sandvig & Sykova, 2016; Lindvall & Kokaia, 2011). The implanted cells have been reported to:

secrete growth and trophic factors, stimulate endogenous neurogenesis (inclusive of the migration and differentiation of these neural progenitors in the injury site), promote angiogenesis, and reduce inflammation. Each of these directly impacts on the penumbra to reduce infarct volume.

In more recent years, collective efforts have been made to improve cell transplantation for acute neural injuries, by the inclusion of biomaterials to restore tissue architecture, support transplanted and residual host neurons whilst also delivering trophic cues to influence inflammation, angiogenesis and neurogenesis. These will be described in greater detail below.

Stimulating endogenous neurogenesis in stroke

In addition to cell transplantation, targeted at replacing lost neurons and/or providing paracrine effects to protect penumbral tissue, is the possibility of stimulating endogenous repair. Within the adult brain, two discrete niches, the subventricular zone of the lateral ventricle and the dentate gyrus of the hippocampus, provide a pool of slowly dividing stem cells. Following stroke, these niche sites upregulate their production of stem cells, with new cells migrating into the injured tissue, Figure 1. Reports are conflicting as to whether these new progenitors are capable of differentiating into functionally integrated neurons, appropriate to the tissue in which they reside, or whether they act as trophic support cells (Arvidsson, Collin, Kirik, Kokaia & Lindvall, 2002; Kokaia & Lindvall, 2012; Parent, 2002; Wright, 2016; Zhang et al., 2010). Nevertheless, it is recognised that the rate of turnover of these new cells is insufficient to promote repair. Consequently, strategies to augment the neurogenic response to a level that may be therapeutically relevant, inclusive of promoting migration of neuroblasts into the injured tissue and their neuronal differentiation, could see self-repair strategies for brain repair. A number of studies have improved the level of injury-induced neurogenesis by delivery of growth factors, morphogens, and inflammatory-modulating molecules – see reviews (Lindvall & Kokaia, 2011; Lindvall & Kokaia, 2015). However, delivery approaches are not trivial in the context of necrotic injuries. The lack of substratum makes efforts to deliver and maintain protein expression at the primary injury site and adjacent penumbra a challenge. Added to this, many recombinant proteins rapidly diffuse away from the site of delivery, and are often rapidly degraded, thereby requiring continual infusion (such as via cumbersome pumps and catheters that risk blockage). Viral vectors require delivery into the already vulnerable penumbra, risking further damage, as well as risks of off-target gene expression and the associated concerns of persistent and/or elevated delivery, beyond the necessary therapeutic window, that could impart negative effects on regeneration. Hence, while various proteins can enhance neurogenesis new strategies to control temporal and spatial delivery could significantly increase the potential of endogenous neurogenesis-based repair.

In the following sections we present an overview of the many types of biomaterials and how they may be employed to promote neural repair by: rebuilding tissue architecture, supporting implanted and residual host cells (inclusive of promoting stem cell proliferation, differentiation, migration, and plasticity), stimulating neurogenesis, and delivering functional proteins.

Biomaterials as Tissue Engineering Scaffolds

Tissue engineering scaffolds have been fabricated from both organic and inorganic materials that have been engineered to interface with specific biological systems (application dependent) to advance regenerative outcomes. In the context of cell-based therapies for neural repair, the hope is that biomaterials will overcome or reduce many of the challenges currently impeding the field. These bioengineered scaffolds are rationally designed to restore tissue architecture where required,

provide physical support for cells (during differentiation and/or implantation), as well as deliver relevant trophic cues to new and residual host cells with spatial and temporal control, Figure 2. To achieve such goals, it will be important to identify materials that are stable, biocompatible/minimally immunogenic, and easy to deliver *in vivo*. Neural tissue engineering exploits scaffold design, material selection, and scaffold morphology to synergistically direct and control cell survival, proliferation, differentiation, and/or integration of neural cells – whether they be host, or graft-derived.

While there is a wide array of biomaterials used as tissue engineering scaffolds, electrospun and hydrogel materials are the most common. Electrospinning is performed by dissolving the intended polymer in an aqueous solution, subsequently applying an electric charge as the solution is slowly dispensed out of a needle. The electrostatic repulsion the occurs as charge accumulates on the solution droplet at the end of the needle is enough to overcome surface tension and causes a thin stream of solution to jet out towards a grounded collector. The solvent evaporates during flight and a sheet of polymer nanofibres is formed, Figure 3Ai. Electrospun nanofibrous scaffolds provide an ideal simulation of neural tissue as fibre alignment (Figure 3Aii-iii), diameter, and interfibre distance can be regulated to generate a surface permissive for neural cell adhesion and axon support (Nisbet et al., 2007; Nisbet, Rodda, Horne, Forsythe & Finkelstein, 2009; Nisbet, Yu, Zahir, Forsythe & Shoichet, 2008). By way of example, we have shown, with poly-ε-caprolactone (PCL), that these nanofiber scaffolds can enhance cell survival, differentiation, and/or axon growth *in vitro*, as well as influence both host- and graft-derived neurons in the intact brain (Horne, Nisbet, Forsythe & Parish, 2010; Nisbet, Rodda, Horne, Forsythe & Finkelstein, 2009; Wang, Forsythe, Nisbet & Parish, 2012). However, the bulkiness of these scaffold sheets makes them difficult to deliver *in vivo* and are consequently more popular for use *in vitro*, or for application in nerve repair. In peripheral nervous system and spinal cord injury, the bandaging/wrapping potential of electrospun materials can be used to form a cylindrical nerve conduit (Schaub, Johnson, Cooper & Gilbert, 2016). Also, aligned fibres (formed using a rotating or oscillating collector during electrospinning) can provide directional guidance to cells. When poly-L-lactic acid (PLLA) nerve conduits were grafted into a 3 mm thoracic spinal cord gap in rats, electrospun conduits allowed infiltration of endogenous cells and the gaps were closed, noting that the aligned PLLA material saw the longest axonal regeneration compared to randomly oriented electrospun fibres and a non-electrospun film (Hurtado et al., 2011).

Hydrogels are 3D networks of hydrated polymers. As the two main components of the extracellular matrix (ECM) are nanofibrous proteins and proteoglycan hydrogels (Frantz, Stewart & Weaver, 2010), these two biomaterials are each well suited to mimic the extracellular environment. Numerous groups have fabricated a variety of hydrogel-based biomaterials targeted at modelling the native 3-dimensional neural environment, repeatedly demonstrating that neural progenitors/neurons cultured on these scaffolds adopt more *in vivo* like morphologies, differentiate, and survive longer than those cultured on conventionally coated culture-ware. Many of these biocompatible scaffolds have also been shown to enhance survival and integration of transplanted neural progenitors (Moriarty *et al*., 2018a,(Rodriguez, Nisbet & Parish, 2012).

Hydrogels are often a more attractive option for use in the brain than electrospun nanofibers. Their fluid-like nature and 3D structure render them ideal for filling irregular voids and interfacing with surrounding tissue, and they can provide an aqueous environment in which cells can interact, without disrupting the local paranchyma, figure 3B. Studies have demonstrated that the co-implantation of fetal and human ESC-derived neural progenitors together with matrigel or collagen-based hydrogels can promote the survival and neuronal differentiation of transplanted cells, while additionally reducing host inflammation and overall infarct volume in the stroke injured brain (Jin et al., 2010; Yu et al., 2010; Zhong, Chan, Morad, Kornblum, Fan & Carmichael, 2010). Thinning hydrogels, either thermoresponsive or mechanically shear thinning, are particularly attractive. Thermo-responsive hydrogels change mechanical properties with temperature. For example, xyloglucan is a plant-derived thermoresponsive polysacharide hydrogel that flows readily at 4°C, enabling injection into the paranchyma, but stiffens into a gel at 37°C, Figure 3Bi. Studies have demonstrated that this biocompatible hydrogel is capable of supressing the host inflammatory responses (Nisbet, Moses, Gengenbach, Forsythe, Finkelstein & Horne, 2009; Nisbet, Rodda, Horne, Forsythe & Finkelstein, 2010), and enhancing the integration of fetal neural transplants in rodent models of PD, by influencing neural progenitor survival, differentiation, and axonal plasticity (Wang, Bruggeman, Kauhausen, Rodriguez, Nisbet & Parish, 2016). Similarly, collagen, another thermoresponsive hydrogel has also been reported to dampen the host immune response to encapsulated cells (Hoban, Newland, Moloney, Howard, Pandit & Dowd, 2013; Moriarty, Pandit & Dowd, 2017), while also enhancing their survival and function after transplantation (Moriarty, Cabre, Alamilla, Pandit & Dowd, 2018; Moriarty, Pandit & Dowd, 2017). Physical hydrogels (those with non-covalent crosslinks) can undergo shear thinning, in which the application of shear stress (such as that applied during injection via needle) causes the hydrogel to flow readily, and stiffen into a gel once the stress is removed. These mechanisms are appealing for neural implantation because cells can be mixed into the liquid hydrogel just prior to injection. On injection, the fluid flows to fill extracellular spaces in the brain, filling irregular voids (where required) and interfacing fully with surrounding tissue, then promptly self-assembling within minutes into a stiffer gel that can be tailored to match the mechnical properties of the brain, and support surrounding host, or newly implanted cells. Such materials (inclusive of hyaluronic acid and collagen gels) have been shown to support both fetal and human PSC-derived progenitors *in vitro* and cell engraftment in PD and stroke models (Adil et al., 2017; Jin et al., 2010; Moriarty, Cabre, Alamilla, Pandit & Dowd, 2018; Moriarty, Pandit & Dowd, 2017; Yu et al., 2010; Zhong, Chan, Morad, Kornblum, Fan & Carmichael, 2010) see also reviews (Jendelova, Kubinova, Sandvig, Erceg, Sandvig & Sykova, 2016; Moriarty & Dowd, 2018; Moriarty, Parish & Dowd, 2018).

An additional promising sub-class of tissue engineering scaffolds are self-assembling peptide (SAP) hydrogel scaffolds that have the advantagous physical and morphological attributes of both electrospun scaffolds and hydrogels (Rodriguez, Parish, Nisbet & Williams, 2013), Figure 3C. These materials are formed from specifically engineered peptide molecules that sponteneously self-assemble into supramolecular nanofibrous structures. This forms a hydrogel network thats offers physical support mimetic of the natural ECM. The design of SAPs initially focused on promoting self-assembly, for instance the use of repeating small and alternating hydrophobic/hydrophilic amino acid sequences in RADA16 to encourage β-sheet interactions, to achieve the physically supportive nanofibre structures (Cormier, 2013). These physical hydrogels are also shear-thinning, meaning they readily flow as liquids when shear stress is applied, and return to a stiffer gel when the force is removed (Rodriguez, Parish, Nisbet & Williams, 2013). More recently, SAPs have been further

engineered to provide biological support as well, by including relevent protein epitopes within the self-assembling peptide sequence (Rodriguez, Parish, Nisbet & Williams, 2013). The importance of specific biological sequences in SAPs was confirmed by comparing the fibronectin epitope arginyl-glycyl-aspartyl (RGD) to a biologically inert but structurally equivalent scrambled sequence (DGR), which resulted in 60% less cell viability when employed as a substrate *in vitro* (Modepalli et al., 2014). In addition, these semi-synthetic materials are xenogeneic-free, inherently less batch-variable (compared to many *in vitro* and *in vivo* used products such as matrigel and geltrex) and degrade into predictable metabolites.

The potential of these SAP scaffolds is only now becoming fully appreciated by neural regenerative researchers. Whilst biologists have invested significant amounts of effort to understand the proteins and genes that underpin direct differentiation and graft integration, notably less time has been spent understanding the function of the extracellular matrix molecules. By way of example, laminins, the major ECM proteins in the brain and once thought of as merely cell adhesion molecules, are now receiving increasing attention due to their influences on stem cell maintenance, survival, differentiation, and plasticity (Theocharidis, Long, ffrench-Constant & Faissner, 2014). Of more specific relevance here, laminin has been shown to promote survival of DA neurons during murine development (by suppressing the cell death associated protein, PTEN), and DA differentiation (by increasing expression of genes critical for VM DA identity, inclusive of LMX1A and PITX3) (Zhang et al., 2017). Added to this, recent protocols for the specification of human PSCs into restricted neuronal fates (inclusive of cortical and dopaminergic) have highlighted the benefit of culturing on laminin substrates (Kirkeby et al., 2017; Lancaster et al., 2013; Niclis et al., 2017a).

In light of this, we, and others, have explored fabricating SAPs that present epitopes for the major components of the brain's ECM, namely fibronectin and laminin. These studies have demonstrated that the laminin-based amino acid sequences isoleucine-lysine-valine-alanine-valine (IKVAV, Figure 3Ci) and tyrosine-isoleucine-glycine-serine-arginine (YIGRS) as well as the RGD fibronectin epitope are capable of positively influencing the survival, proliferation, and differentiation of both fetal and/or human PSC-derived dopaminergic and cortical progenitors *in vitro* as well as following transplantation (Rodriguez et al., 2017; Rodriguez et al., 2014; Silva et al., 2004; Somaa et al., 2017). Interestingly, the presentation of these short epitopes has been shown to enhance neuronal adhesion, differentiation, and axonal growth of neural progenitors with greater efficiency than the full-length proteins themselves, due to the high density of signals that can be presented (Silva et al., 2004). Most recently implantation of this IKVAV-SAP together with human ESC-derived cortical progenitors have been shown to induce functional recovery in stroke models, superior to cell grafts alone, by mechanisms including increased graft survival/size and neuronal differentiation. These new neurons, in the presence of the laminin-based SAP, displayed more maturity, reflected in their morphology and electrophysiological function. Interestingly, not only did this tissue-specific biomaterial support the implanted cells, but greater angiogenesis and reduced secondary degeneration were also observed (Somaa et al., 2017), contributing to enhanced motor function recovery.

Hydrogels and electrospun materials can also be used together in composite biomaterials to achieve the benefits of both material classes. However, the composites inherently combine the material drawbacks as well, which often renders them unsuitable for injection and more common in spinal cord injury. A composite of aligned poly ε-caprolactone-co-ethyl ethylene phosphate electrospun and collagen hydrogel materials was used to treat spinal cord injury in rats. The materials also

incorporated controlled neurotrophin delivery, resulting in aligned axon regeneration from endogenous cells (Nguyen, Gao, Lin, Wu, Wang & Chew, 2017). To maintain the desirable injectability of hydrogels within a hydrogel/electrospun composite, the electrospun scaffold can be cut into loose, short fibres that can then be mixed within hydrogels. This technique has recently been used to create a composite of electrospun PCL fibres within a thermoresponsive xyloglucan hydrogel for use in a rodent model of Parkinson's disease, where it was shown to improve the integration of fetal tissue grafts (Wang, Bruggeman, Kauhausen, Rodriguez, Nisbet & Parish, 2016). A similar composite of PLA fibres within an IKVAV SAP hydrogel has also been developed and tested *in vitro* (Bruggeman, Wang, Maclean, Parish, Williams & Nisbet, 2017). Both of these short fibre composite materials were also used to provide controlled growth factor delivery and are discussed further in the relevant section below.

Scaffold biodegradation

In addition to considerations surrounding the biocompatibility of biomaterials employed in tissue repair are issues pertaining to the longevity of presentation requirements and/or impact following scaffold degradation (Maclean, Rodriguez, Parish, Williams & Nisbet, 2016). It is often assumed that biodegradable scaffolds will be most advantageous, given the perception that the long-term presence of a foreign body into the CNS will be invasive and may cause additional tissue damage(Maclean, Rodriguez, Parish, Williams & Nisbet, 2016). However, employment of biodegradable scaffolds requires specific materials engineering design to ensure that cytotoxic molecules are not released during scaffold degradation. In these instances, biomaterials such as self-assembling peptides may be most optimal, as they degrade into non-toxic amino acids, that can provide the added benefit of being metabolically utilized by adjacent cells (Ellis-Behnke et al., 2006).

It also remains critical that the rate of degradation will allow maintained structural integrity, such that the scaffold supports the host tissue and/or implanted cells for sufficient duration to avoid collapse and loss of functional capacity. The question of whether a biodegradable or non-biodegradable material should be used in repair will depend on the type of neural injury. There have been many pre-clinical spinal cord injury studies where filling the injury cavity alone has resulted in improved outcomes (see review - (Nomura, Tator & Shoichet, 2006)). In this context a non-degradable scaffold that provides a bridging network for the support of axonal regeneration may be more optimal. The requirements for stroke are similar - where decreasing the lesion volume can result in significant functional improvement through the support of both endogenous and grafted cells (Nisbet et al., 2018; Somaa et al., 2017; Tuladhar, Payne & Shoichet, 2018). In the context of more discrete injuries such as PD and HD, a scaffold that provides physical and chemical support for the survival and differentiation of cells upon implantation, but then slowly degrades once the cells have appropriately integrated within the host circuitry, may be a desirable alternative. As such, it is clear that the development of scaffolds for cell therapy should be tailored to meet the specific needs and requirements for each given neural injury.

Support during injection

In addition to the physical support offered by biomaterial scaffolds *in situ*, they can also provide support and protection during delivery. Specifically, hydrogels have been used to reduce damaging shear forces experience by cells during injection, figure 4. The laminar flow dynamics of Newtonian liquids (which includes saline solutions and media, typically used in current clinical procedures (Foster, Marquardt & Heilshorn, 2017)) during injection result in varying flow rates, with the highest

flow rate in the middle of the needle (Amer, Rose, Shakesheff, Modo & White, 2017). This results in cell stretching as the cells experience different velocities (Chisti, 2001). These shear forces are believed to cause rupture in cell membranes, which, along with shear stress and high pressure, causes cell death post-injection (Aguado, Mulyasasmita, Su, Lampe & Heilshorn, 2012; Foster, Marquardt & Heilshorn, 2017). Hydrogels, and in particular shear-thinning hydrogels, can ameliorate these effects by providing a more consistent flow profile throughout the needle during injection (Amer, Rose, Shakesheff, Modo & White, 2017). By way of example, a chitosan-based shear thinning hydrogel was shown to significantly increase survival of rat neural stem cells 1, 3, and 5 days post-injection through a 21-guage needle (Wei, Zhao, Chen, Zhang & Zhang, 2016). This is particularly applicable to the transplantation of vulnerable cell types, such as DA progenitors, which experience significant cell death after implantation (Boonman, 1999; Zawada, 1988).

Utilizing biomaterials to deliver trophic support *in vitro* and *in vivo*

In vitro, a cocktail of small molecules and proteins are supplemented into the culture media to promote neural induction and regional specification into lineage-restricted neuronal subpopulations such as midbrain dopamine or cortical progenitors. *In vivo*, numerous proteins such as neurotrophins, morphogens, chemokines, and axon guidance molecules have been demonstrated to influence survival, differentiation, and plasticity of new and residual stem cells (Bruggeman, Williams & Nisbet, 2018; Chilton, 2006; Moshayedi et al., 2016; Rodriguez et al., 2017; Tayalia & Mooney, 2009). However, even under optimal conditions, differentiations result in heterogeneous populations inclusive of proliferating progenitors, immature and mature neurons of the desired phenotype, as well as off-target populations. *In vivo*, survival, differentiation, and plasticity also remain below optimal levels. Underpinning these outcomes is likely the lack of stability of many of these proteins - with basic fibroblast growth factor (bFGF) having an elimination half-life of 40 min (Edelman, 1993; Lazarous et al., 1997), whilst the half-life of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) *in vivo* are similarly short, at just 45 mins and 2hours, respectively (Bruggeman, Rodriguez, Parish, Williams & Nisbet, 2016; Krewson & Saltzman, 1996). Consequently, despite frequent (commonly daily) media and protein changes, differentiating stem cell cultures are exposed to extreme dose fluctuations that likely underpin the asynchronistic and heterogenic differentiations.

Added to this, in the broader field of drug delivery, systemic administration (e.g. intravenously) is the norm and targeted delivery from the bloodstream is one of the most significant areas of research focus – but using biomaterial scaffolds as localised drug reservoirs bypasses this issue. In the central nervous system, this localisation also means bypassing the blood brain barrier (BBB), which can inhibit the delivery of large or hydrophilic molecules from the bloodstream (Stockwell, Abdi, Lu, Maheshwari & Taghibiglou, 2014). Currently, direct and ongoing protein/drug delivery to the brain is achieved by intracerebroventricular (Paul et al., 2015) or intraparenchymal catheters (Tuladhar, Payne & Shoichet, 2018), which are invasive and ongoing, and cause iatrogenic injury. Delivery of viral vectors, to sustain protein delivery from transduced cells, has also been explored, however, it presents further challenges in controlling the temporal and spatial delivery, as well as dose. Consistency in delivered dose is an especially important factor to consider because actual fluctuations are not always clear from drug delivery data as conventionally displayed. Cumulative release profiles inherently fail to show the large natural variability of the *in situ* delivery rate . In this regard, incorporation of drugs/proteins/small molecules into biomaterials, targeted for *in vitro* and *in vivo* use, provides means for more tightly controlled (sustained and consistent) release. Methods

of protein incorporation within biomaterials and examples of their application, in the context of neural repair are described below.

Encapsulation of proteins within biomaterials

Hydrogel materials are very well suited to providing diffusion-based protein delivery, with the exact release profile dependent on the hydrogel mesh size, degree of swelling, and electrostatic properties, as well as the properties of the protein being delivered. Proteins can also be mixed directly into electrospun materials through solution or emulstion electrospinning, when the protein is soluble or insoluble respectively in the original polymer solution (Faccendini et al., 2017). Surprisingly, neither the organic solvent conditions of the polymer solution nor the electric current applied during electrospinning generally affect protein bioactivity. Insoluble proteins remain in aqueous phase regions of the emulsion when electrospinning (Faccendini et al., 2017) and in the resulting firbes (Liu et al., 2018b), and the actual current involved in electrospinning is so low that electrospinning directly onto living tissue is a safe practice (Yan et al., 2016). NGF and GDNF have been emulsion electrospun within several polymers (poly-L-lactide-co-caprolactone (PGLA), poly-lacto-co-glycolic acid, poly-D, L-lactic acid (PDLLA)), with bioactivity confirmed by increased neurite outgrowth and neural differentiation of PC12 cells *in vitro* (Li, Su, Liu, Tan, Mo & Ramakrishna, 2010; Liu et al., 2018b). Aligned PLA fibres were emulsion electrospun with 6-aminonicotinamide (6AN); the material demonstrated a 2-week sustained release and concomitantly reduced astrocyte activity (effect of 6AN) and directed dorsal root ganglia axonal outgrowth (effect of fibre alignment) (Schaub & Gilbert, 2011). A drawback of this technique is that emulsion electrospinning can affect material properties of the fibre scaffold, including fibre diameter and alignment (Johnson, D'Amato & Gilbert, 2016).

Simple mixing of proteins into hydrogels generally provides a more sustained delivery than an injection of protein in solution. Loading soluble BDNF or epidermal growth factor (EGF) into hyaluronic acid (HA) hydrogels was shown to improve iPSC-derived NPC survival *in vitro* (Moshayedi et al., 2016) and promote endogenous NSC proliferation in an animal model of stroke (Cooke, Wang, Morshead & Shoichet, 2011; Wang, Cooke, Sachewsky, Morshead & Shoichet, 2013). GDNF mixed directly into a collagen hydrogel with primary dopaminergic neurons resulted in dramatic increases in dopaminergic cell survival and host tissue reinnervation (Moriarty, Cabre, Alamilla, Pandit & Dowd, 2018; Moriarty, Pandit & Dowd, 2017). We recently demonstrated the benefit of delivering BDNF within an IKVAV SAP hydrogel to promote the survival and differentiation of human ESC-derived cortical progenitors in a stroke model, also demonstrating increased vascularisation of the graft and protection of the host tissue compared to BDNF delivery in the absence of the scaffold (Nisbet et al., 2018). A limitation of this approach, however, is that the exact duration and level of delivery are dependent on the interactions between the specific protein and the specific material – and are not inherently optimised. As such, more involved methods are required to tightly control delivery profiles. Several of the most prevalent designed protein delivery systems from biomaterials are discussed below.

Covalent and Affinity Immobilisations of proteins onto biomaterials

Immobilisation delivery systems primarily focus on providing sustained and/or localised delivery. In these systems, proteins are attached to the biomaterial scaffold permanently or semi-permanently as their diffusional release is significantly slowed. The attachment can be achieved via covalent bonding, chemical crosslinkers, and/or strong affinities between biomolecules. Covalent bonding,

with a chemical crosslinker, has been used to immobilize proteins such as BDNF and GDNF onto the nanofibers of electrospun scaffolds, resulting in sustained presentation over several months (Wang, Bruggeman, Sheean, Turner, Nisbet & Parish, 2014; Wang, Bruggeman, Kauhausen, Rodriguez, Nisbet & Parish, 2016; Wang, Forsythe, Nisbet & Parish, 2012). These functionalised scaffolds enhanced the survival, proliferation, differentiation, and/or plasticity of rodent neural progenitors *in vitro* and following *in vivo* implantation (Rodriguez et al., 2017; Somaa et al., 2017; Wang, Bruggeman, Kauhausen, Rodriguez, Nisbet & Parish, 2016; Wang, Forsythe, Nisbet & Parish, 2012). This is an effective method to provide long-term presentation of extracellular signalling molecules that do not need to be internalised by cells to function. In this context, the duration of presentation becomes dependent on the material degradation timeframe.

Immobilisation via affinity interactions is more common, as it requires minimal or no chemical modification of the protein of interest and can allow a controlled level of diffusion out of the material. Heparin binding is commonly used to achieve this, whereby a biomaterial is functionalised with heparin in order to semi-immobilise any of the heparin binding growth factors – inclusive of platelet-derived growth factor (PDGF), vascular endothelial growth factor, FGF, transforming growth factor beta (TGT-β), and the neurotrophin families (Martino, Briquez, Ranga, Lutlolf & Hubbell, 2013). In a rodent stroke model, such a heparin modified scaffold (a hyaluronic acid hydrogel) was used to retain BDNF, in an effort to support the survival and differentiation of transplantated human iPSC-derived neural progenitors (Moshayedi et al., 2016). While the heparin system is very versatile, with so many heparin-binding growth factors, it's versatility is also one of the main drawbacks of the system. While the approach can be used for many proteins, it cannot be used to independently tune the release profiles of multiple proteins since each would have a release profile dictacted by the heparin in the biomaterial. In the aforementioned stroke study, the relative concentrations of two growth factors were optimised before *in vivo* testing, but there was no attempt to independently control their release (Moshayedi et al., 2016). The prevelance of heparin binding among natural ECM proteins can also present complications *in vivo*, when host ECM proteins are abundant (Tuladhar, Payne & Shoichet, 2018). Consequently, newer stategies are being developed to provide more specific heparin binding, including the use of heparin fractions with protein-specific affinity (Wang et al., 2014). Other affinity pairs (e.g. streptavidin-biotin, barnase-barstar) can also be used. A methacrylate-chitosan hydrogel with covalently immobilised streptavidin mixed with biotinylated growth factors interferon-γ (IFN-γ) and PDGF was tested in a hemisection spinal cord injury in rats, and showed increased neuronal and oligodendrocyte differentiation respectively of implanted NSPCs (Li et al., 2016). Using multiple affinity immobilisation pairs within a single material can allow spatial and dose control of multiple proteins (Wylie, Ahsan, Aizawa, Maxwell, Morshead & Stoichet, 2011), but the temporal control is still limited. Immobilisation systems all work by stopping or slowing diffusion, but cannot provide sequential release.

Protein Delivery Vehicles

Using distinct protein delivery vehicles within tissue engineering scaffolds presents another protein delivery option. Nanoparticles are the most common delivery vehicle. An advantage of nanoparticle systems is the ease of their incorporation into both hydrogel and electrospun materials, they are mixed in directly like proteins, but with added layers of control to delivery. Layers, specifically, are the most common method of controlling the delivery profile from nanoparticles. Particles are formed with different proteins in different layers, or with the addition of shielding layers, to provide additional diffusional barriers and delay release. A dual particle system, consisting of 1) PEG particles

containing EGF; and 2) layered particles containing EPO in the core, shielded by an outer particle layer, were mixed into a hydrogel and used in a mouse stroke model to enhance endogenous neurogenesis. Here they demonstrated the capacity of EGF to promote stem cell proliferation, with subsequent, rather than simultaneous, EPO increasing neuroprotection – effects that were superior to direct protein infusion (Wang, Cooke, Sachewsky, Morshead & Shoichet, 2013). The layered control mechanism can also be used with electrospun materials, using layered sheets of electrospun fibres containing different proteins. Layers of NGF and GDNF loaded PDLLA were used to provide sequential delivery of growth factors *in vitro* (Liu et al., 2018a). A similar layered electrospun material has been used to delivery neurotrophin NT-3, BDNF, and PDGF in a rodent model of crushed sciatic nerve. It was found that fast delivery of NT-3 and BDNF followed by slow delivery of PDGF yielded the greatest nerve recovery (Hong, Hong, Pang, Lee, Yi & Koh, 2018). Interesting in this study was the use of layering to independently optimise material properties and protein delivery, with an aligned fibre PCL layer used as the outermost cell-interfacing layer, and random PLGA fibres used in underlying protein reservoir layers (Hong, Hong, Pang, Lee, Yi & Koh, 2018).

Nanoparticles can also be used in encapsulation-free protein delivery systems. Here electrostatic surface interactions were sufficient to bind growth factors to the surface of polymer nanoparticles, allowing the particles to act as delivery vehicles while protecting the protein growth factors from the harsh chemical conditions involved in encapsulation (Pakulska, 2016). This system requires complimentary surface charges between the polymer and the protein being delivered. While many proteins have been encapsulated and delivered without harm from the chemical process, this encapsulation-free study is particularly interesting as it highlights how easily proteins can be immobilised by physical electrostatic. These interactions must be considered in other protein delivery and biomaterial scaffold systems, as they may affect the *in-situ* delivery profiles.

Recently, short fibres cut from electrospun scaffolds have been prepared as protein delivery vehicles in electrospun-hydrogel composite materials. PCL fibres with covalently immobilised GDNF in a xyloglucan hydrogel showed enhanced integration of cell grafts in a rodent model of PD (Wang, Bruggeman, Kauhausen, Rodriguez, Nisbet & Parish, 2016). Emulsion electrospun PLA fibres loaded with GDNF achieved a 1-week delay in delivery *in vitro* when mixed into an IKVAV SAP hydrogel, while not interfering with sustained release from the hydrogel component of the composite (thus allowing temporally distinct delivery of multiple growth factors from a single material) (Bruggeman, Wang, Maclean, Parish, Williams & Nisbet, 2017). These short fibre systems have potential benefits over nanoparticle systems in that they also provide the electrospun nanofibre structure that has been shown to support neural cells.

Conclusion

Recent research into the use of stem cells for neural repair has branched out in several directions: optimising the cell populations used in the graft, providing appropriate support to the graft during and after implantation, and more sophisticated protein delivery systems to provide trophic support. These are very diverse fields, with topics ranging from nanotechnology in biomaterial scaffold development to cell biology in the investigation of stem cell differentiation to fluid dynamics in the assessment of shear forces experienced during injection. While significant progress has been made in all of these directions, there are still gaps in the efficacy of stem cell treatments. Truly effective stem cell strategies will require a combined approach from all of these avenues of advancement – which is not a trivial matter. It has been noted in this review that many of the protein delivery

strategies discussed affect the properties of the biomaterial scaffold used. This presents an obstacle to the concomitant optimisation of physical and trophic support. Similarly, we have recently discovered (unpublished) that scaffolds providing optimal physical support during injection via needle do not necessarily provide the best support post-transplantation, and vice versa. Protein delivery systems are predominantly at the stage of single-protein delivery, and when multiple proteins are delivered the dose and timing is not optimised. Results are often described in terms of significant improvements rather than optimisation or achieving ideal conditions.

Part of this is a paucity of knowledge as to what defines the optimal conditions. There is a significant gap between *in vitro* and *in vivo* results, and limited understanding of how the varying aspects of a biomaterial or stem cell therapy methods affect each other. There has been some work using the design of experiments approach to more systematically address this problem and assess/optimise many variables at once (Moshayedi et al., 2016), and more work like this will certainly advance the field. Stem cell therapy is a multi-faceted topic, and the vastness of the future directions is itself a step forward. The field has progressed significantly in identifying more areas that impact on stem cell therapies, which will ultimately allow for significant improvement in these methods. There are many active avenues of research in the field, and exciting advancements being made.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017).

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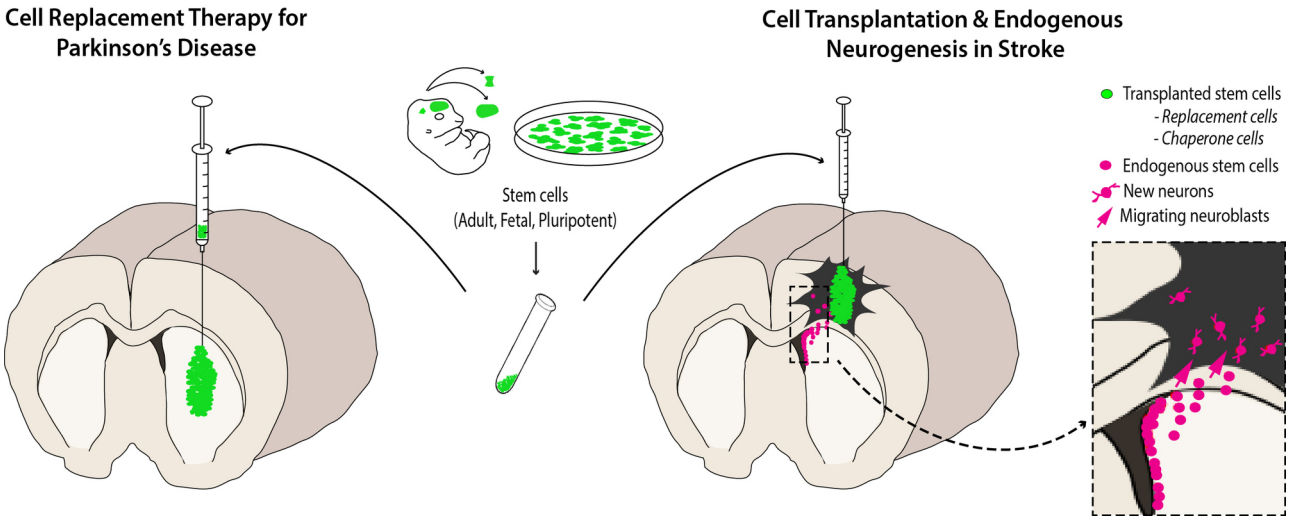
FIGURE LEGENDS

Figure 1: Stem cell-based therapies for neural repair. Stem cells/neural progenitors (green), isolated from fetal tissue, can be transplanted directly into the injured host tissue, illustrated here in a model of Parkinson's disease and stroke. As an alternative, more standardised and sustainable cell source, pluripotent stem cells (requiring in vitro differentiation prior to in vivo delivery) are increasingly being studied in tissue repair. Studies have demonstrated the capacity for these transplanted cells to survive and functionally integrate, replacing neurons lost to the primary injury. Transplanted cells can also act as chaperone cells to support surrounding tissue. Alternatively, quiescent stem cells, present within discrete locations within the host brain (magenta), can be mobilised to replace neurons (endogenous neurogenesis) and/or deliver trophic cues, targeted at reducing injury and promoting repair.

Figure 2: Applications for biomaterials in neural repair. Following disease or injury, biomaterials can be used to rebuild tissue architecture, they can be blended together will cells and/or incorporate functional proteins, small molecules and drugs. By providing physical and chemical biomimetics of the neural environment, these biomaterials can enhance the survival, differentiation and plasticity of neural cells in vitro, as well as following transplantation. These attributes can also contribute to enhanced endogenous neurogenesis. Hydrogel scaffolds can provide additional benefits of reducing shear forces exerted on cells during injection into the host tissue.

Figure 3: Biomaterials employed in neural repair. (Ai) Electrospun scaffolds are fine nanofibrous meshes formed by the axial stretching of a viscoelastic polymer solution under an applied voltage. Modifications in synthesis can result in scaffolds sheets with (Aii) aligned or (Aiii) random fibres that can influence cellular differentiation and neurite growth. (Bi) Hydrogels are microporous scaffolds that consist of hydrophilic polymer chains interspersed in water. Thermoresponsive hydrogels can be biologically advantageous for in vivo delivery; being liquid at one temperature (e.g. 4°C) yet gelling at another (37°C). (Bii) Transmission electron microscopy (TEM) image of a xyloglucan hydrogel. (C) Self assembling peptide scaffolds result from non-covalent intermolecular forces that result in the formation of organised scaffolds. (Ci) Example peptide sequence for a laminin epitope IKVAV. (Cii) Schematic of the organised assembly of peptides. (Ciii) TEM of an IKVAV-self assembling peptide and (Civ) demonstration of its gel properties, under defined pH and shear-force conditions.

Figure 4: Shear forces on cell during injection. Left: cells injected using a hydrogel (Foster, Marquardt & Heilshorn, 2017). Right: forces/flow in a shear thinning hydrogel (Amer, Rose, Shakesheff, Modo & White, 2017).



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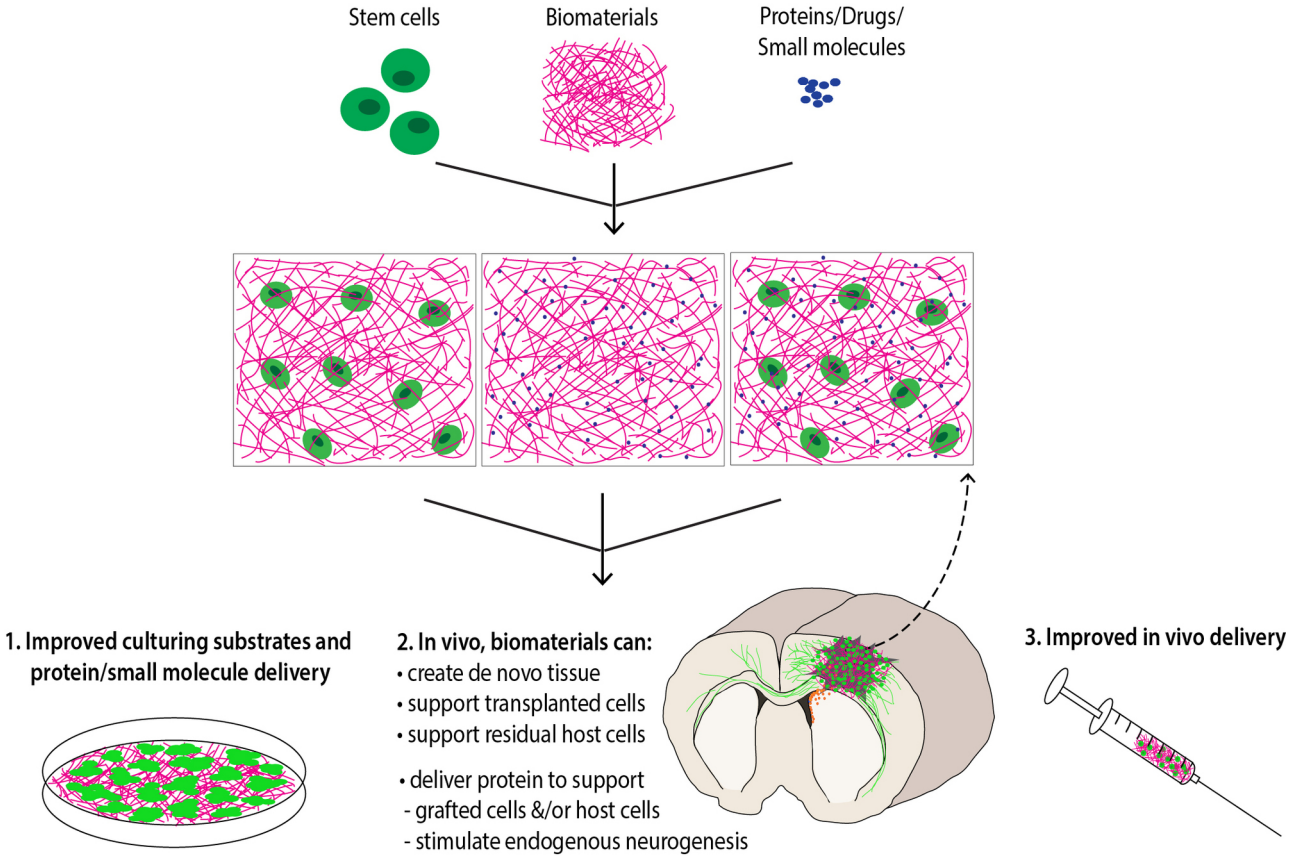

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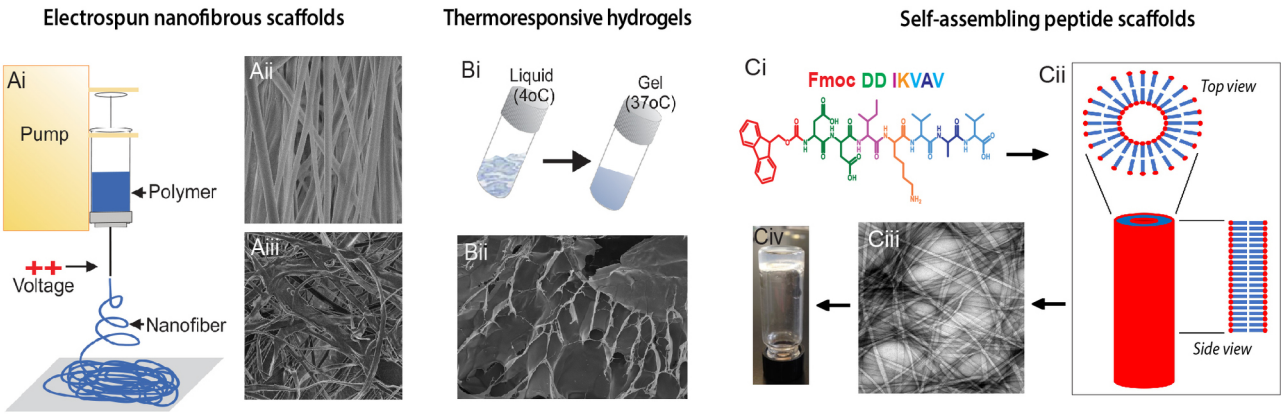

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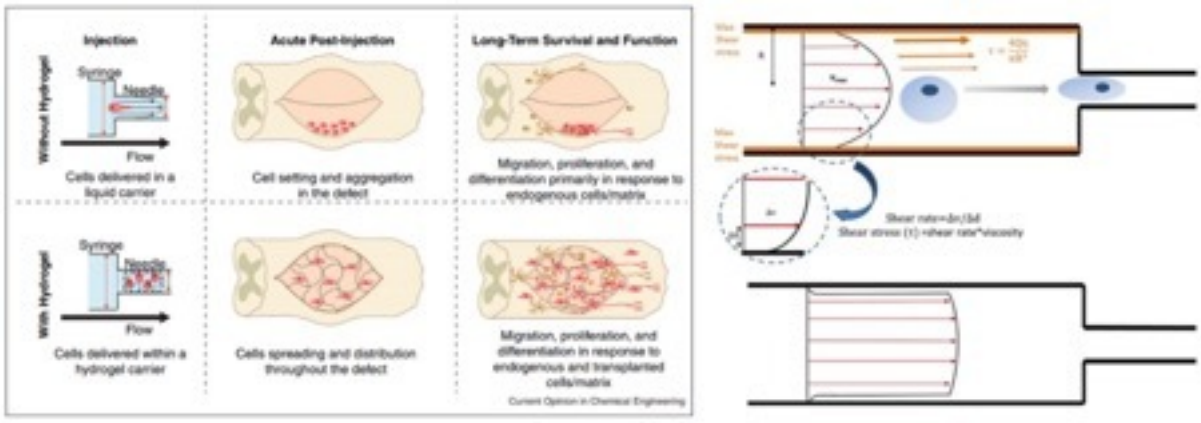

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Hyperlink List

Basic fibroblast growth factor (bFGF) -
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1808

Brain derived neurotrophic factor (BDNF) -
http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=4872

Glial-cell derived neurotrophic factor (GDNF) -
http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=4940

Nerve growth Factor (NGF)
http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5026

Laminin Sonic hedgehog (SHH) (could not find)

Transforming Growth Factor 3 beta (TGF3b) -
http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5062

Bone Morphogenic Protein (BMP) -
http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=960

epidermal growth factor (EGF) -
http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=4916

platelet-derived growth factor (PDGF) -
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1804

vascular endothelial growth factor (VEGF) -
http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=963