Biomimetic material systems for neural progenitor cell-based therapy

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1. ABSTRACT

Reconstruction and regeneration of the central nervous system (CNS) following injury is a formidable task. However, cell replacement with transplanted neural progenitor cells (NPC) is a promising technique that has resulted in various levels of functional recovery in animals that had experienced an experimental injury of the brain or spinal cord. Unfortunately, CNS injury often leads to significant tissue damage and loss, limiting the survival and integration of transplanted NPC. In response, researchers have developed many biomaterial substrates that have been used to culture, transplant, and influence the differentiation and integration of transplanted NPC. Biomaterial scaffolds are a three-dimensional lattice that can be engineered to support NPC in vitro as well as serving as a temporary extracellular matrix (ECM) after transplantation. Scaffold modification with bioactive components, such as proteins, adhesive peptide sequences, and growth factors, allow researchers to modulate NPC responses as well as the local environment of the transplantation site. Biomimetic approaches also can include materials that recapitulate the structural dimensions of the ECM, namely self-assembling nanofibers. These materials can be useful for altering the tissue microenvironment by reducing inflammation and glial scarring, which may further enhance NPC survival and integration into functional neural circuitry. This review describes various biomaterial constructs, with a focus on biomimetic systems that have been used in modulating NPC behavior in culture and/or in transplanting NPC to the CNS.

2. INTRODUCTION

For many years, it has been thought that the adult mammalian central nervous system (CNS) is unable to regenerate following injury or disease. However, in 1992, Reynolds and Weiss reported the existence of stem cells in the adult rat brain (1). Since then the CNS has been shown to have much more plasticity and capacity for regeneration than previously thought, and there have been many papers published describing the brain's potential for self repair as well as repair assisted by transplanted cells (2). In spite of this progress, there is still a lack of clinically effective therapies using transplanted cells for repairing the injured brain and spinal cord in order to restore lost function (3). In part, this lack of effective cell-based therapeutics is due to the complexity of nervous system repair and the challenging environment encountered by cells transplanted to the injured CNS (4).

The potential for using transplanted cells to repair the CNS has lead to much attention being paid to the factors that affect the differentiation of neural progenitor cells (NPC). While NPC have committed to a neural lineage, they have not yet differentiated into neurons or glial cells. Factors mediating NPC differentiation include chemical signals such as cytokines, drugs, and neuropeptides, as well as physical determinants such as extracellular matrix (ECM) proteins and cell-cell contact. These chemotactic and haptotactic stimuli act in concert in a spatio-temporal fashion and influence protein expression that defines the progression of cell differentiation, specification, and migration. As the cell biology of NPC is elucidated, biomaterials engineers seek to incorporate aspects of this knowledge into materials that may direct NPC towards a specific lineage, support NPC growth *in vitro*, and enhance NPC integration and survival after implantation to the CNS.

2.1. Focus of paper

While there are numerous reviews describing various transplantation efforts with NPC and embryonic stem cells (ESC), this review seeks to examine the cellular response to biomaterials and its effects on gene expression and cell differentiation. The focus here will be on biomimetic systems, specifically those that incorporate bioactive peptides and/or bound cytokines into biomaterial constructs for the purpose of neural engineering using NPC. These systems will be analyzed based on their structural and biological components and the associated effects on cultured and transplanted NPC. Transplantation considerations such as inflammation and glial scarring also will be taken into account in discussing optimizing transplant efficacy. The overall goal of this review will be to discuss results obtained thus far in order to assist in formulating new pathways for future research that will involve multiple components and lead to effective strategies for NPC-based therapy.

2.2. Neural progenitor cell biology: sources, identification, and receptors

There exist several sources of NPC: ESC that have been differentiated into a neural lineage, NPC derived from the fetal nervous system, and NPC from the adult brain. Therapies in humans based on adult-derived NPC are limited by the difficulties associated with the retrieval and isolation of adult NPC. For example, harvesting autologous adult human NPC would require invasive procedures in the brain or spinal cord and is not feasible. In view of such problems, researchers have focused attention on two alternative sources of NPC for transplantation to the CNS; neuralized ESC, and NPC from fetal brains.

2.2.1. Embryonic stem cells

During mammalian development all of the tissues of the body are formed from ESC. ESC have two defining properties; self-renewal, and pluripotency (5). ESC can divide almost indefinitely while retaining pluripotency and are therefore an excellent source for generating NPC for CNS transplantation. However, due to the high tumorigenicity of transplanted undifferentiated ESC, it is desirable to differentiate ESC into NPC or mature cell types prior to CNS transplantation. ESC have been isolated from pre-implantation blastocysts (6), expanded into cell lines, and are well-characterized in terms of the media conditions necessary for their specification into neural lineages (7). Recent evidence shows that specific cytokine combinations, as well as the timing of cytokine addition, can influence the specification of ESC into particular subtypes of neural cells (8), illustrating the molecular detail that researchers must understand in order to reliably produce specific cells for transplantation. For example, ESC have been differentiated to a neural lineage *in vitro* and these neuralized cells have been transplanted into the brains of mice where the cells subsequently differentiated into neurons and glia (9, 10).

2.2.2. Fetal and adult neural progenitor cells

Neural progenitor cells are capable of producing neurons, oligodendrocytes, and astrocytes *in vitro* (11) and *in vivo* (12). In the adult brain there are two active sites of neurogenesis; the subgranular zone (SGZ) of the hippocampal dentate gyrus, and the subventricular zone (SVZ), which is located on the lateral wall of the anterior horn of the lateral ventricles (5). However, the destinations of the newly generated neurons produced by these neurogenic regions are very restricted under normal conditions. The SGZ produces new neurons exclusively for the hippocampal granule layer, while the SVZ generates neurons for the olfactory bulb. As a result, endogenous adult NPC are capable of replacing very limited numbers of lost neurons, and the targeted migration in response to injury is minimal (13, 14). Although the SGZ and SVZ are the only proven sites of adult neurogenesis, NPC have been isolated *in vitro* from the adult cerebral cortex (15), cerebellum (16), and spinal cord (17). Thus NPC exist in many areas of the adult CNS and represent a cell population whose regenerative capabilities have not yet been realized. In contrast, the transplantation of exogenous NPC to the damaged or diseased brain for therapeutic purposes has received considerable attention. After transplantation, NPC demonstrate the potential to survive and integrate with host brain circuitry (5, 18-20).

Biomimetic material systems

Table 1. Common molecular markers used to define the identity of neural cells

Antigen	Description
Multipotent Progenitor Cell Markers	
• Nestin	Neural progenitor cell
• GFAP	Astrocyte, radial glia, possibly progenitor cell
• Notch1	Immature cell, SVZ in adult brain
• Sox1/2	Neuroepithelium, neural progenitor cell
• Lex1	Embryonic and adult NPC
• CD133 (prominin-1)	Hematopoietic, NPC, and cancer "stem" cell
• Musashi-1	Neural progenitor, glioma cell
Neuronal Marker	
• Beta III tubulin (TuJ 1)	Immature to mature neurons
• Doublecortin	Newly generated neurons, mature neurons
• Hu	Neuron-specific RNA binding protein
• MAP2	Immature to mature neurons
• NeuN	Immature to mature neurons
• Neurofilament	Immature to mature neurons
• TUC4	Immature postmitotic neuron
• SCG10	Mature neuron, related to regeneration
• GAP43	Neuronal growth cone, related to growth and regeneration
Astrocyte Markers	
• GFAP	Astrocyte, radial glia, SVZ and SGZ progenitor cell
• S100-beta	Astrocyte, ependymal cell
• Vimentin	Glial progenitor, radial glia, reactive and immature astrocyte
• Nestin	Immature astrocyte, NPC
Radial Glial Markers	
• BLBP	Radial glia, multipotent progenitor
• Vimentin	Glial progenitor, radial glia, reactive immature astrocyte
• GFAP	Radial glia, astrocyte, possibly progenitor cell
• GLAST	Radial glia to postmitotic astrocyte
• RC1/2	Radial glia
Oligodendrocyte Markers	
• Myelin basic protein (MBP)	Mature oligodendrocyte myelin
• CNPase	CNS myelin
• MOBP	Oligodendrocyte myelin
• GalC (galactocerebroside)	Mature oligodendrocyte
• Myelin associated glycoprotein	Oligodendrocyte myelin
• PLP/DM20	Oligodendrocyte; integral membrane proteins in myelin
• Claudin11/OSP	Immature to mature oligodendrocyte
• NOGO (neurite outgrowth inhibitor)	Oligodendrocyte
Microglia	
• CD68	microglia
• CD11b	microglia
Commited Progenitor Markers	
• A2B5	Oligodendrocyte progenitor, glial progenitor
• O4	Immature-mature oligodendrocyte, Schwann cell
• PSA-NCAM	Neuronal/glial progenitor

Modified from ref 120

Fetal brains are a common source of NPC for research. Fetal NPC are similar to adult NPC in terms of their trilineage capability, however, recent findings have demonstrated that fetal NPC may be intrinsically different from adult NPC in terms of their differentiative capabilities (21). An apparent intrinsic developmental program is evident in ESC culture in which cells of different ages produce different neural sub-types under identical culture conditions (22). Thus it is possible that the properties of NPC continue to change during development through to adulthood. This potential characteristic of NPC has implications for understanding results obtained using NPC derived from animals of different ages, as well as in designing appropriate culture systems and transplantation paradigms.

A critical aspect of NPC research is the reliable identification of specific differentiated cell types. This is done by identifying cell-specific proteins that are expressed during different stages in the process of differentiation (Table 1). The unambiguous identification of NPC can be problematic *in vitro* where heterogenous cultures are the inevitable product of asymmetric division. While nestin is an intermediate filament protein expressed in undifferentiated NPC, nestin expression by itself does not unequivocally define a cell as a multipotent NPC because nestin expression has been observed in postmitotic cortical cells (24), reactive and immature astrocytes, radial glia, and skeletal muscle (25, 26). Moreover, some NPC harvested from brain parenchyma often co-express nestin and glial fibrillary acidic protein, a marker commonly associated with astrocytes (23).

2.2.3. NPC-material interaction

Proper physical contacts are necessary for the survival and functional integration of transplanted NPC. Integrin receptors coordinate cell-ECM and cell-material attachments that regulate NPC migration and growth and

are thus involved with the intracellular transduction of information concerning the cellular environment. ECM proteins also play a role in the migration of NPC by facilitating the transient binding required for migration. *In vivo,* however, NPC migration involves more than just ECM contacts. Chemoattractants and chemorepellants, e.g. netrins (27, 28) and slit proteins (29) respectively are required to direct migrating NPC to their final destination. For example, NPC migrating from the SVZ to the olfactory bulb along the rostral migratory stream secrete slit proteins that can act in a paracrine or autocrine fashion, indicating the intrinsic capability of NPC to coordinate their migration (29). NPC also control their migration by expressing the polysialylated form of the neural cell adhesion molecule which mediates transient adhesions.

Many cell types, including NPC, have been shown to orient themselves preferentially along the axis of surface textures (30), further illustrating the importance of cell-substratum interactions. Interestingly, time spent in culture can affect NPC competence and/or differentiative potential, possibly through reprogramming or de-differentiation (31). This observation alone demonstrates the necessity for well-characterized cell-material interactions to prevent cells from becoming 'lost' in a sea of unnatural signaling. Therefore, biomaterial engineers have created materials that mediate specific cellular responses, including cell-material adhesions that serve in defined culture systems and may also support the survival of transplanted NPC.

2.3. Role of materials in NPC research and application

Mitogenic growth factors, neurotrophins, and other cytokines are known to exert potent effects on stem and progenitor cell fate while the effects of physical interactions are less clear. For example, the differing effects of suspension and adherent cultures on cell differentiation have not been fully resolved. Recently, Nat *et al* (32) compared mRNA levels of key genes in human ESC during neural differentiation in suspension and adherent cultures in the absence of serum and did not find any significant difference. However, some workers maintain that NPC differentiate more readily when bound to culture substrates than when suspended in neurospheres.

NPC activity does vary in response to different materials (33), which can mediate disparate signaling events. Therefore, cells receive instructive signals from both physical and chemical sources *in vivo*, and the goal of biomaterials engineers is to produce culture systems that can provide a similar level of dual signaling. Unfortunately, the effects of material contacts on NPC differentiation are still poorly understood. From the time that NPC are retrieved from either embryonic or adult brains, contact with foreign materials has largely unknown effects on cellular phenotype. Adherent NPC cultures are in constant contact with a culture substrate which may consist of simple tissue-culture polystyrene (TCPS), TCPS coated with a proteins or synthetic polymers, or a three-dimensional lattice. Each of these substrate platforms may elicit different cell receptor and contact-mediated signaling events that cells use to interact with their environment. To accommodate these interactions, substrate material design parameters will need to be customized for various intended uses, e.g., *in vitro* conditions to promote cell proliferation and restrain differentiation, conditions to induce differentiation, or conditions to prepare cells for transplantation.

2.3.1. Culture substrates

In recent years, surfaces on which NPC are cultured have progressed from TCPS alone to surfaces that mimic the natural ECM environment in an effort to optimize NPC expansion and differentiation. While TCPS is a substrate that supports NPC adhesion, proliferation, and differentiation, it does so only through physical, contact-mediated signals. TCPS can be coated with proteins, and purified ECM proteins such as fibronectin and laminin have been used extensively as substrates for NPC culture. Cellular attachment, proliferation, and neurite growth are all enhanced on these proteins in comparison to TCPS alone. Laminin has been shown to have higher activity than fibronectin in promoting neurite growth, migration, and proliferation of human cortical progenitors (34), while cerebellar progenitor cells migrate similarly on both protein substrates (33). In the latter study, laminin was shown to promote neuronal and glial differentiation of cortical progenitors at levels that exceed those seen with fibronectin and Matrigel^{TM}, an effect that the authors attributed to a higher level of NPC survival on laminin. These differences in the migration of cells and in the growth of neurites on different substrates are typical of cells isolated from different CNS regions and are commonly attributed to differences in integrin expression profiles and associated integrin signaling.

2.3.2. Transplantable scaffolds

Experimental methods to alleviate neurodegenerative disorders or injuries to the brain typically involve the injection of cell suspensions into the damaged or diseased brain in an effort to replace dead or dysfunctional cells with transplanted cells. Cell replacement strategies have been used in models of Parkinson's (35) and Alzheimer's (36) where the goal is the replacement of lost neurotransmitter function by transplanted cells able to secrete either dopamine, for Parkinson's, or acetylcholine, for Alzheimer's. Keirstead *et al* (18) used oligodendrocyte precursor suspensions to remyelinate spinal axons that had been damaged from a systematic weight drop injury. Other CNS trauma such as stroke or spinal cord injury results in varying amounts of irretrievable tissue loss, and even the most competent transplanted cells may not be capable of replacing the structural complexity of intact tissue. Biomaterial-based tissue engineering therapies for replacing substantial tissue loss ideally could provide the structural integrity of the lost tissue as well as the appropriate cell type, number, and distribution.

Biomaterial systems designed as scaffolds that can support NPC adhesion, growth, and differentiation *in vitro* and after implantation are highly desirable for the reconstruction of damaged neural tissue. While providing important attachment sites for transplanted cells, cell

Table 2. Materials used in NSC-based research

Material	Applications
Natural	
• laminin	cell adhesion, coating
• fibronectin	cell adhesion, coating
• Matrigel	culture substrate, can incorporate into materials
• hyaluronic acid	hydrogel with conjugated PDL for brain tissue repair (47)
• collagen I	3D culture matrix, supports neuronal differentiation (43)
• collagen IV	combined with PHEMA, Schwann cell transplant. (62)
• Alginate`	direct axonal regrowth in spinal cord (38)
• chitosan	attach YIGSR, IKVAV in tube for spinal nerver repair (82)
• fibrin	3D scaffold for directing ESC to neural lineage (40)
• gelatin	amenable to peptide modification, can crosslink
Synthetic	
• poly(lactic acid) (PLA)	attachment to PEG to increase cellular affinity (65)
• poly(glycolic acid) (PGA)	cell scaffold for transplantation (51), attach to PEG (65)
• poly(lactic-co-glycolic acid)(PLGA)	cell scaffold (52), cytokine release from microparticles (54)
• poly(L-lactic acid) (PLLA)	culture substrate (55, 56)
• poly(D,L-lactic acid) (PDLLA)	nerve guidance channel (61)
• self-assembling peptide nanofiber	culture scaffold, can attach peptide (93-95)
Hydrogels	
• poly(ethylene glycol) (PEG)	cell scaffold, attachment of peptides/polymers (63-65)
• poly(hydroxyethyl methacrylate) (PHEMA)	with collagen and cell scaffold (62), prevent adhesion in vitro
• poly[N-(2-hydroxypropyl) methacrylamide] (PHPMA)	attachment of RGD, spinal cord repair (73)
Peptide sequences	
• RGD	many ECM proteins: cellular adhesion, migration
• PHSRNG6RGD	fibronectin: promote cellular adhesion, migration beyond RGD
• IKVAV	laminin: weak cell adhesion
• IKVAV nanofiber	cell scaffold, selective neuronal differentiation (95)
• YIGSR	laminin: cell adhesion
• PDSGR	laminin: cell adhesion
• PRGDSGYRGDS	collagen VI: cell adhesion
• SKPPGTSS	bone marrow homing protein-1: tight adhesion, neurite spreading
• PFSSTKT	bone marrow homing protein-2: tight adhesion, neurite spreading
Others	
• polylysine	Crosslinked with PEG (63), adhesive coating for cell culture
• poly(D-lysine)	attached to HA hydrogel to promote cell adhesion (47)
• TCPS	standard polystyrene culture flask material

scaffolds can facilitate neural reconstruction by filling the space left by necrotic tissue. Along with the appropriate chemical and mechanical characteristics, scaffold materials that present bioactive moieties to the cells may provide another tool for instructing the phenotypic development of NPC. Thus technologies have progressed from TCPS to a biomimetic strategy entailing combinatorial material approaches that involve multiple conjugated peptides and bound growth factors. This biomimetic approach seeks to recapitulate key aspects of the native tissue environment in an attempt to provide signals to cells that increase cellular integration and enhance transplant effectiveness. Future biomimetic systems also might be capable of creating functional neural networks *in vitro* for neural tissue engineering.

3. BIOMATERIALS DEVELOPMENT

Suitable biomaterial scaffolds ideally possess the following attributes: they are capable of modification and design, exhibit controlled biodegradation, have good cytocompatibility, are able to enhance specific cell-material interactions, are non-immunogenic, can be produced and purified efficiently, and are chemically stable in vivo. The modification and design of materials is important since unmodified materials have limited efficacy, and therefore materials engineering is needed to tailor biomaterial properties for specific applications. For example, biodegradation should be controlled temporally to coincide with new tissue formation while facilitating the ingrowth of host tissues. Degradation of synthetic scaffolds into bioresorbable products can avoid possible long-term host inflammatory responses that are commonly associated with implanted biomaterials. Cytocompatibility is essential; cells must interact favorably with a scaffold that serves as a temporary ECM. Enhancing specific cell-material interactions is critical when designing a biomaterial scaffold for application in a specific tissue. Transplanted cells may be required to adhere, proliferate, secrete trophic factors, migrate and extend neurites within the ECM template. Biomaterials also must elicit a minimal immune response since infiltrating immune cells may hinder the integration of transplanted cells, especially in CNS repair where astroglia often aggregate to form a barrier to growth (37). Scaffold materials should have chemical stability in aqueous solutions under physiological conditions in order to maintain proper mechanical properties and structure after transplantation. The three-dimensional structure of a scaffold affects cell migration, proliferation, and differentiation of NPC. This includes porosity, fiber thickness and density, and the degree of cross-linking or branching of the polymers employed. These are interrelated properties; by increasing the degree of cross-linking, fiber density increases causing a decrease in porosity. The material properties mentioned above vary from material to material and affect the cellular response dramatically. Table 2 provides a list of materials that have been used in NPC-based research.

3.1. Natural materials

Naturally occurring materials can be used for tissue engineering and cell replacement strategies due to their inherent biological activity and porous structure. Natural hydrogels such as alginate and chitosan have demonstrated *in vivo* biocompatibility and have been used in tissue engineering (38, 39). Recently, fibrin scaffolds were used as substrates for murine ESC culture and were shown to promote neurite growth and ESC differentiation into neurons and glia (40). Alginate hydrogel materials continue to show promise in various areas of neural research such as supporting NPC expansion within alginate beads (41), or promoting axonal regrowth in the injured spinal cord (38). Alginate polymers can be modified to include bioactive peptides (42), which makes them an attractive material for neural tissue engineering.

Collagens are the main structural protein of the ECM and are capable of forming a hydrogel while promoting cell attachment, and can, therefore, be useful when incorporated into scaffolds (43, 44). Collagen properties can be altered via crosslinking chemically with gluteraldehyde, enzymatically with transglutaminase, or photocrosslinked with UV light (45). Recently, cortical NPC were dispersed within a type I collagen scaffold and were shown to proliferate actively and differentiate into neurons. These neurons initially were cholinergic and purinergic, but upon maturing their neurotransmitter profiles changed into GABAergic and glutamatergic (43). Hyaluronic acid (HA) is also abundantly expressed throughout the body and is an important component of the brain ECM and plays a critical role during neural development (46). HA has been combined with poly-D-lysine (PDL) to form a hydrogel copolymer (HA-PDL) and used for brain tissue repair (47). PDL residues were covalently linked to the HA backbone which provided a positively charged surface that increased protein adsorption and allowed for neural cell ingrowth and axonal extension. The HA-PDL materials elicited a transient and localized inflammatory response that decreased sharply after three weeks as assessed by low levels of glial and macrophage staining in the vicinity of the transplant. At this time, the ingrowth of new blood vessels was observed and the HA-PDL had largely degraded.

Despite their advantages, natural materials may contain residual growth factors and undefined and/or non-quantified constituents (48). Moreover, natural materials are less amenable to modification compared to synthetic materials, which are cheaper and more easily characterized. Thus, researchers have focused on characterizing NPC behavior on biocompatible synthetic materials, some of which have been engineered to contain some of the bioactive moieties of natural materials.

3.2. Synthetic materials
3.2.1 PLA, PGA, PLGA, PLLA

An important class of biomaterials used for cell scaffolds is poly(α-hydroxy esters). This class includes poly(glycolic acid)(PGA), poly(lactic acid)(PLA), and the copolymer; poly(lactic-co-glycolic acid)(PLGA). These are synthetic polymers that are readily made into three-dimensional scaffolds that biodegrade via bulk hydrolysis. The degradation end products are CO_2 and H_2O and these materials are therefore termed 'bioresorbable'. One can control the mechanical properties by varying the relative amounts of PGA and PLA in the PLGA copolymer (49). Poly(α-hydroxy ester) materials are mechanically strong and have been approved by the FDA for tissue engineering (50), however, these materials are not readily modified and are processed under toxic conditions, which limits the ability to incorporate trophic factors and cells into the bulk of the construct.

Snyder *et al.* (51) used a woven array of PGA fibers seeded with immortalized neural cells (C17.2) in an attempt to reconstruct a massive lesion of the brain produced by ischemia-hypoxia. The PGA fiber diameter was reported to be 10-15 μm and the scaffold had an overall bulk density of 64.7 mg/ml. In culture, C17.2 cells grew exuberantly on the PGA scaffold, displaying adhesion with extended neurites and positive staining for the neuronal marker neurofilament in >90% of the cells. Following transplantation, there was evidence of interactions between host and donor-derived cells and some host neurons appear to have sent projections to the damaged area of the brain, suggesting integration with the scaffold.

Teng *et al.* (52) used a PLGA copolymer scaffolding material seeded with C17.2 cells to repair traumatic spinal cord injury. The PLGA used was 50/50 PLA/PGA, and the scaffold was comprised of 75% PLGA (40 kDa) and 25% block copolymer of PLGA (30 kDa) and polylysine. The construct was designed to mimic the natural structure of the spinal cord and consisted of an inner porous layer and an oriented outside layer, made from the same material but through different processes. Similar to PGA, C17.2 cells attached and grew throughout the scaffold in culture prior to transplantation. Following transplantation, animals with the transplanted cell scaffolds scored significantly higher on the Basso-Beattie-Bresnahan (53) open field walking scale compared to animals transplanted with cells alone. Surprisingly, the authors noted that the transplanted cells did not express neurofilament, that most of them expressed nestin, and that their addition to the scaffold did not lead to improved functional recovery compared with the scaffold by itself. Moreover, a small proportion of the transplanted cells became astrocytes, shown by their expression of glial fibrillary acidic protein, which is an undesirable event that can add to the glial scar often associated with CNS injury. However, since only a small proportion of grafted cells differentiated into astrocytes, the authors concluded that their methods did not contribute to scar formation.

Mahoney and Saltzman (54) used PLGA (50:50) microparticles for the transplantation of fetal brain cells. The particle diameters were in the range of 0.5-5 μm and were used to encapsulate nerve growth factor (NGF). The PLGA microparticles were then coated with polylysine to enhance cell adhesion and microparticle aggregation in order to form 'neo-tissues', which were approximately 170 μm in diameter. Mahoney and Saltzman termed this neo-

tissue a 'programmable microenvironment' because NGF release could be controlled by altering either the number of particles transplanted or the rate at which the microparticles degrade. A common way to control degradation time with PLGA is by changing the relative amounts of the two polymer constituents. The time required for complete degradation of PLA can be on the order of years, while PGA degradation is rapid and can occur within weeks depending on porosity and concentration. The highest rate of NGF release by the microparticles *in vivo* occurred over the first week following implantation with a rapid decrease of concentration for up to 21 days. In response to released NGF, the transplanted cells differentiated into cholinergic neurons, based on their production of choline acetyltransferase. This method of growth factor delivery could be useful in treating neurodegenerative diseases and injured CNS.

Poly(L-lactic acid)(PLLA) has been explored as a possible neural engineering substrate. Yang *et al.* have thoroughly characterized NPC adhesion and neurite extension on PLLA nanofibers (55) and electrospun PLLA (56). In the nanofiber study, PLLA (100 kDa) was prepared using phase separation which allows material porosity and fiber diameter to be controlled by varying PLLA concentration. Nanofiber PLLA attempts to mimic ECM collagen structurally to promote NPC attachment and subsequent neurite extension. This was achieved after one day in culture, yet the number of adherent cells was slightly lower than on traditional TCPS. The electrospinning technique allows for control over fiber diameter and alignment. Yang *et al.* [56] found that NPC grew preferentially along the fiber oriented axis, and cell proliferation and neurite length was greatest on the aligned nanofiber substrate compared to the aligned microfiber substrate and substrates with random fiber orientation.

Poly(D,L-lactide)(PDLLA) is a type of poly(alpha-hydroxy ester) and has been sufficiently characterized to show that it is cytocompatible, non-immunogenic, and biodegradable *in vivo* (57). PDLLA has been used in a wide range of applications including a cell culture surface (58), a cellular scaffold for transplantation (59), and a coating to improve host-material interaction (60). Patist *et al.* (61) used PDLLA guidance channels impregnated with brain-derived neurotrophic factor (BDNF) for spinal cord repair (61). This modified biomaterial resulted in the generation of 20% more neurons and twice as many blood vessels in the BDNF scaffold compared to controls; however axonal regeneration was low. The researchers suggested that the same material could be seeded with Schwann cells to help myelinate axons.

3.2.2. Hydrogel systems

Hydrogels are a macromolecular network of crosslinked hydrophilic molecules or polymers that readily saturate with water and therefore represent a good physiological model of the ECM. Hydrogel scaffolds are used extensively for tissue engineering scaffolds because they typically are degradable, have mechanical and structural properties of many biological tissues, are amenable to modification, and can be processed under mild conditions which allows for cell encapsulation. Depending on the degree of hydrophilicity, protein adsorption to the hydrogel surface will be inhibited, and thus cellular interactions will be decreased. For this reason, synthetic hydrogels provide researchers with a tool for controlling specific cellular interactions via hydrogel modification. These modifications typically involve the conjugation of various molecules such as proteins, peptide sequences, growth factors and other cytokines, drugs, and/or additional polymers. Synthetic hydrogel materials are more easily modified with bioactive moieties than are the natural hydrogel materials (fibrin, alginate, collagen, and HA), thus providing researchers with more flexibility in their material design.

Alternatively, the simple addition of purified ECM proteins to hydrogel scaffolds can confer the necessary bioactivity. Plant *et al.* (62) transplanted a Schwann cell-seeded poly(2-hydroxyethyl methacrylate) (poly-HEMA) hydrogel modified with collagen IV into a lesion of the rat optic tract. These materials preferentially supported the integration of transplanted Schwann cells and the regrowing axons were myelinated by those cells. Ford *et al.* (63) created a macroporous polyethylene glycol (PEG)-based hydrogel conjugated with poly-L-lysine for the co-culture of neural progenitors and endothelial cells. This was accomplished by reacting the polymeric mixture around a PLGA sponge, which was subsequently hydrolyzed in NaOH, producing a hydrogel architecture similar to the PLGA. PEG-based hydrogels also have been used for the controlled release of bioactive factors, such as vascular endothelial growth factor, to promote angiogenesis (64).

Recently, Mahoney and Anseth [65] photo-encapsulated cells (using Irgacure® 2959 as a photoinitiator) within a PEG hydrogel with conjugated PLA and PGA. This material was used to culture a heterogeneous cell population from E14 rat forebrain and demonstrated that these cells survived and formed aggregates that had a delayed neurite extension occurring at day 12 of culture. The neural cells expressed markers of all three neural lineages and secreted fibronectin, yet the material did not appear to allow for cellular migration or the formation of neural networks (65). While photoencapsulation is capable of producing three-dimensional cellular scaffolds that could serve as transplantation vehicles for NPC, exposure to UV radiation and photoinitiator radicals may have unpredictable cellular effects.

To investigate these variables we assayed the survival of NPC derived from the SVZ of E15 rat embryos while varying UV exposure and intensity (Figure 1). NPC were plated 24 hours prior to UV stimulation, exposed to UV, returned to the incubator for twenty four hours, and then counted. Survival percentages shown in Figure 1 are relative to cell counts taken before UV irradiation. There was sustained cell proliferation under all conditions except the highest level of UV exposure; 26 mW/cm^2 for 3 min. The effects of the photoinitiator, Irgacure® 2959, were
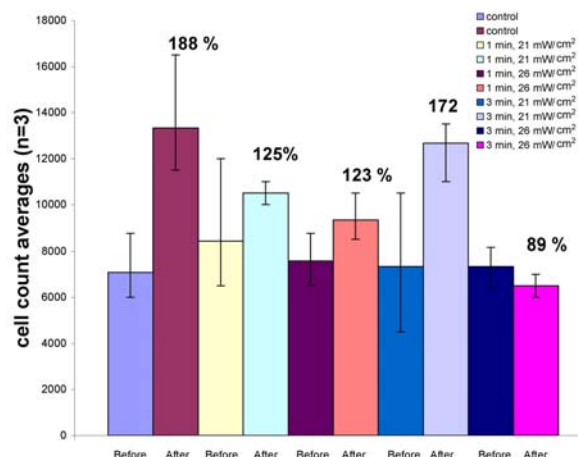
Figure 1. UV toxicity on NPC. Effects of long spectrum UV on E15 SVZ-derived NPC survival. Irradiation was conducted immediately following the 'Before' cell count, cells were then returned to the incubator for 24 hrs followed by the 'After' cell count. Percentages are relative to the 'Before' count in each condition and demonstrate that proliferation was retained in NPC following all UV conditions except the most extreme (26 mW/cm² for 3 min). Error bars are standard error of the mean (n=3).
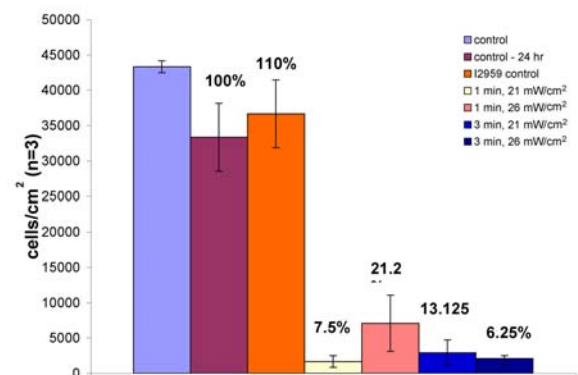

Figure 2. Effects of Irgacure®2959 on NPC. NPC cultures were exposed to UV in the presence of Irgacure®2959 photoinitiator. Blue box: Is a control cell count prior to UV exposure. Purple box: Is the control condition at 24 hrs. The other conditions are detailed in the legend for which cell counts were obtained 24 hrs post UV exposure.

determined under identical exposure conditions (Figure 2). Results indicated that E15 NPC were very sensitive to Irgacure® 2959 photoinitiation since all experimental conditions resulted in a dramatic decrease cell viability (Figure 2). Although we evaluated the effects of UV irradiation and exposure to Irgacure® 2959 separately, the addition of PEG-diacrylate to create a hydrogel did not affect cell survival. The disparity in cell viability between this study and that of Mahoney and Anseth (65) is not surprising since different cell types display varying levels of photoinitiator sensitivity (66). This sensitivity is appears to be positively correlated with proliferation rate, as demonstrated by the fact that rapidly proliferating rat SVZ-

derived NPC, which differ from cortical progenitors, are very susceptible to photopolymerization.

3.3. Material modification: biomimetic approaches

NPC receive instructive cues from chemical and physical sources which affect differentiation and growth. Therefore, by conjugating specific bioactive molecules/peptides onto the culture surface, researchers can control the signals that cells receive from external sources. One logical design of materials for tissue engineering applications involves ECM recapitulation through the incorporation of adhesive peptide sequences and cytokines into material constructs. Common features of biomimetic scaffolds are peptides bound to linker molecules that in turn are bound to the scaffold material, and the incorporation of soluble or bound growth factors. Figure 3 depicts an example biomaterial that presents these factors as well as containing stable (PEG) and degradable (gelatin) components, thus providing controlled biodegradation. Biomimetic approaches have the added advantage of bioactivity, which can promote biologically correct cell adhesion within a well-defined system, thereby allowing researchers to identify the effects of various signals.

3.3.1. Modification with ECM-derived peptides: RGD, IKVAV, YIGSR and PHSRNG₆RGD

Cellular adhesion to ECM molecules is mediated by a family of cell surface receptors called integrins. Integrins are heterodimeric transmembrane proteins that directly connect the extracellular environment to the intracellular actin cytoskeleton thus facilitating intracellular signaling pathways and cellular migration. Since integrins are crucial mediators of cell adhesion, they have become targets for tissue engineering strategies that seek to incorporate the integrin recognition sequences of ECM molecules. Integrins bind selectively to specific adhesion peptide sequences located on ECM proteins, although multiple integrins can bind to the same peptide sequence. Binding of integrins induces dimerization of the alpha and beta subunits causing a conformational change and phosphorylation of the intracellular domain leading to signal cascades and cytoskeletal remodeling.

Fibronectin is a large multidomain glycoprotein that can be found on cell surfaces, within the ECM, in plasma, or other body fluids. The adhesive tripeptide arginine-glycine-aspartic acid (RGD) was first identified in fibronectin (66), but is now known to be incorporated in a variety of ECM proteins including collagen, vitronectin, thrombospondin, von Willebrand factor, fibrinogen, and laminins (67). RGD has been used extensively in tissue engineering applications including the modification of biomaterials and tissue grafts (68), biodegradable scaffolds for cartilage (69) and dermal wound repair (70), matrices for hepatocyte transplantation (71), the promotion of cellular ingrowth in vascular applications (72), and facilitation of axonal outgrowth in the injured spinal cord (73). The alpha5beta1 integrin receptor is known to selectively bind RGD. Its intracellular amino terminus modulates actin assembly (74), and therefore influences cellular migration. Decreases in alpha5beta1 integrin
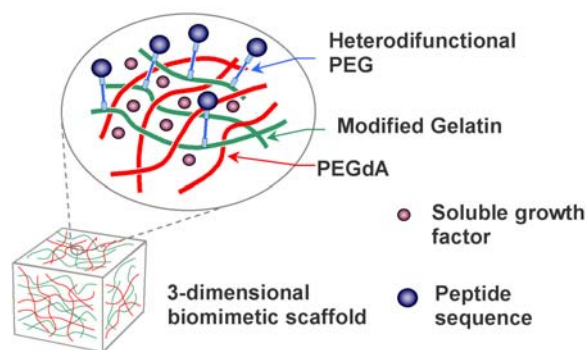
Figure 3. Biomimetic ECM. The close up view displays the key components that can be incorporated into biomimetic material scaffolds; bound peptide sequences, soluble or bound growth factors and/or other cytokines, degradable component (e.g. gelatin), and a stable backbone (photo-crosslinked PEG).
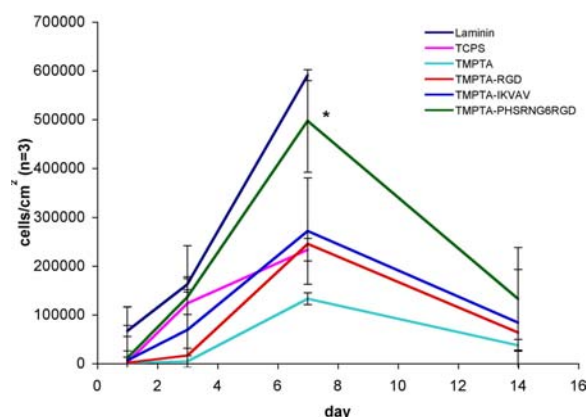

Figure 4. E15 SVZ-derived NPC adhesion and proliferation on peptide-engrafted surfaces. PHSRNG$_6$RGD supported significantly higher (*) adhesion and proliferation over all other peptides (p>0.05), except IKVAV. The laminin condition reached confluency by day 7. The decrease in cell counts at day 14 was due to clusters of NPC disengaging from the surface prior to counting. This indicates that NPC adhered less tightly to peptide-engrafted surfaces than to laminin-coated coverslips. NPC on laminin-coated coverslips reached confluency by day 7.

expression correlate with increased differentiation of cortical progenitors (75).

Beta1 integrins as a class contribute to NPC self-renewal via the mitogen-activated protein kinase pathway (76), and are required for NPC migration (77). Woerly *et al.*, attached RGD to a poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA) hydrogel and implanted it in adult and developing rat spinal cords (73). The group reported that the transplanted hydrogel facilitated host cell infiltration, angiogenesis and axonal growth and a reduction of necrosis compared to controls. Most of the surviving axons were myelinated, which was attributed to host Schwann cell migration into the porous scaffold.

The laminins are a family of heterotrimeric proteins that are widely expressed throughout the body and are key substrates during embryonic development (78). The identified cell binding domains on laminin consist of the peptide sequences Ile-Lys-Val-Ala-Val (IKVAV), found on the alpha chain, and Tyr-Ile-Gly-Ser-Arg (YIGSR), which is found on the beta chain. The YIGSR sequence is thought to facilitate cell binding (79) while the IKVAV peptide facilitates neurite extension (80). Yu and Shoichet (81) conjugated both of these laminin peptide sequences to a copolymer hydrogel of poly(2-hydroxylethyl methacrylate) and poly(aminoethyl methacrylate) (p(HEMA-co-AEMA)). They observed the average neurite extension length of cultured dorsal root ganglion neurons was the same on the peptide surface and laminin positive control, indicating that these peptide sequences can recreate the binding affinity of laminin. Itoh *et al.* (82) bound YIGSR and IKVAV into a chitosan tube for spinal cord regeneration and observed that these peptides resulted in similar tissue histology as did the laminin protein tube condition.

In addition to that mentioned above, fibronectin also contains a peptide sequence Pro-His-Ser-Arg-Asn (PHSRN) that acts synergistically with RGD to enhance cell binding (83). In an effort to mimic fibronectin more completely, we have investigated the PHSRNG$_6$RGD peptide which contains both fibronectin peptides with a hexa-glycine spacer that approximates the distance between them on the native protein. Using E15 NPC from rat SVZ we assayed cellular proliferation and adhesion on RGD, PHSRNG$_6$RGD, and IKVAV peptide-engrafted surfaces (Figure 4). The PHSRNG$_6$RGD peptide supported the highest level of NPC adhesion (p>0.05) and approached the levels of adhesion observed on laminin-coated TCPS. This result, showing that the synergistic peptide motif, PHSRN, confers a greater level of adhesion than RGD or IKVAV alone, demonstrates the efficacy of more complete recreation of ECM components in biomaterial constructs.

A similar study reported by Aota *et al.* (84) found that NPC adhesion and proliferation levels varied in a dose-dependant manner on RGD surfaces, and RGD concentrations of 21 pmol/cm^2 supported adhesion levels that exceeded those on laminin. Interestingly, when Aota *et al.* examined the binding domain IKVAV, they found that the adult hippocampal NPC did not adhere or extend neurites. Moreover, using culture conditions that promote neuronal differentiation by incorporating retinoic acid and forskolin, Aota *et al.* found that beta-III tubulin and GFAP mRNA levels were similar in NPC grown on RGD and laminin suggesting these surfaces were equally permissive for glial and neuronal differentiation. However, these mRNA levels were dramatically decreased in cells grown on IKVAV surfaces.

3.2.2. Cytokine and growth factor release/delivery

While cytokine delivery, including biomaterial-mediated release, to the CNS is a well-established technique, there is a relative paucity of attempts using material-mediated cytokine release in conjunction with NPC transplantation. Nevertheless, cytokine delivery from

materials is a useful biomimetic approach and the technologies discussed here may be applied to NPC-based therapies. Traditionally, biomaterials have been impregnated with growth factors that diffuse into host tissue upon implantation. Appropriate biodegradation facilitates this release and has been useful in the delivery of BDNF, a neuroprotective molecule, from collagen tubes (85) and from poly(D,L-lactic acid) scaffolds (61). NGF stimulates neurite outgrowth and cell survival, and has therefore been an attractive cytokine for delivery. *In vitro*, NGF has been tethered to polyethylene oxide-polypropylene oxide-polyethylene oxide triblock copolymers and adsorbed to cellulose acetate fibers to create a three-dimensional NGF culture system (86). This system allowed a much higher expansion of the embryonic germ layer-derived cells when compared with controls. However, the bound NGF signaling appeared to be less potent than soluble NGF.

PLGA has been used to encapsulate NGF for sustained release *in vitro* (87) and *in vivo* (54, 88, 89). In a recent report, hydrogels comprised of PLA-PEG-PLA triblock copolymers were used to photoencapsulate ciliary neurotrophic factor, BDNF, and neurotrophin-3 (90). The PLA component allowed the hydrogel to degrade, and cytokine release profiles were characterized.

Cytokine delivery also can be accomplished by engineering the transplanted cells to over-express a given cytokine. Whitlesey and Shea (91) used PLG microspheres to encapsulate liposomal-NGF DNA (lipoplex) that was then used to transfect PC12 cells. This produced cells that secreted high levels of functional NGF which resulted in increased neurite growth of co-cultured dorsal root ganglion neurons. These examples demonstrate the wide variety of techniques that can be used to deliver growth factors and provide a means of controlling release profiles within transplants.

3.3.3. Nanofiber scaffolds

Researchers have attempted to recreate the physical dimensions of the ECM through nanofiber scaffolds that are a three-dimensional meshwork formed through the non-covalent self-assembly of fibers with nanometer diameters. Self-assembly is a hallmark of biological systems and therefore synthetic self-assembling materials provide yet another tool to try to mimic the ECM. Zhang *et al.* (92) developed a scaffolding material that self-assembled from amphiphilic oligopeptides. The oligopeptide units consist of alternating positively (lysine or arginine) and negatively (aspartate or glutamate) charged residues separated by hydrophobic residues (alanine or leucine). This scaffold material, termed RADA16, was used to test neuronal growth using rat hippocampal neurons. The growth of these cells in culture accompanied by functional synapse formation was observed (93).

Using the RADA16 nanofiber as a base material, Gelain *et al.* (94) conjugated a host of bioactive peptides including RGDS (fibronectin), PRGDSGYRGDS (collagen VI), IKVAV, YIGSR, PDSGR (laminin), as well at two bone marrow homing peptides SKPPGTSS (BMHP1) and

PFSSTKT (BMHP2). Adult mouse NPC were cultured on these surfaces and assayed for adhesion level, viability, and differentiation. Interestingly, the BMHPs supported greater adhesion than all other peptides. The BMHPs seemed to induce differentiation nonspecifically as there were approximately equal proportions of neurons, glia, and undifferentiated cells at seven days in culture.

Silva *et al.* (95) engineered a nanofiber material that self-assembled from amphiphilic molecules that contained the laminin peptide IKVAV at one end. This produced nanofibers that were literally composed of the IKVAV epitope and this high density was purported to selectively differentiate the NPC into neurons. The nanofibers produced a gel-like three-dimensional structure that was highly porous and hydrated. The IKVAV peptide also promoted axonal outgrowth, and the neuronal differentiation on the IKVAV nanofibers significantly exceeded that of NPC cultured on laminin and PDL substrates. Silva *et al.* [95] did not mention whether these nanofibers were biodegradable, and unfortunately there have been no reported transplantation studies conducted with these materials.

4. CLINICAL CONSIDERATIONS OF NPC-BASED THERAPY

The therapeutic application of NPC-based technologies will require both an in depth understanding of NPC biology and the signaling environment of the transplantation site. This information will be crucial *in situ*ations where there has been extensive tissue damage such as following stroke, cerebral hemorrhage, or neurodegenerative disorders. In stroke victims, sudden ischemic insult can cause massive cell death via necrosis, which immediately elicits an inflammatory response. Although inflammation is part of the body's natural progression towards healing, inflammation may hinder therapeutic interventions, especially cell transplantation. Glial scarring during end-stage neural healing, caused by the migration and proliferation of astrocytes at the injury site, also inhibits regenerative efforts. Thus, researchers are presented with a relatively narrow window of efficacy and an inhospitable environment into which cells may be transplanted. These factors will be discussed in the following sections, along with additional considerations facing clinicians and researchers in optimizing transplant efficacy.

4.1. Inflammation

Nervous system injury, whether it is traumatic brain injury or spinal cord injury, involves an initial direct damage followed by a secondary inflammatory cascade that further contributes to tissue damage and cell loss. Following injury, microglia and astrocytes migrate to the site and secrete pro-inflammatory cytokines such as interleukins -1beta and -6 (IL-1beta, IL-6) and tumor necrosis factor (TNF), which act on NPC by inhibiting differentiation thus blocking neurogenesis (96). Interestingly, TNF-alpha can increase NPC proliferation by blocking neurogenesis (97). IL-6 is particularly relevant to transplantation efforts since it known to promote

gliogenesis and increase apoptosis (98). This might explain why many transplanted cells differentiate into glial cells and not neurons. Treatment with anti-inflammatory drugs, specifically minocycline, has been shown to decrease tissue loss and partially restore locomotor function following spinal cord injury (99, 100). Glucocorticoids, including dexamethasone, a glucocorticoid receptor agonist, have been administered as anti-inflammatory molecules (101). Similar to NGF release described earlier, PLGA nanoparticles have been used to release dexamethasone (102). Glucocorticoids, however, were later shown to inhibit neurogenesis (103) which is undesirable in neural cell replacement applications.

While glial cells are primarily responsible for inflammatory cytokine production, it has been shown that at some point during the healing of an injury, all neural cells, including neurons, produce inflammatory cytokines, including TNF and IL-1beta (104). Recently, researchers assayed the possible beneficial effects of transplanting NPC with concomitant immunosuppression. Human NPC were transplanted into a unilateral lesion of the corpus striatum created by 6-hydroxydopamine, which is a standard model for Parkinson's disease. No significant difference in the survival of transplanted cells was found between immunosuppressed groups and controls, although there was only mild activation of astrocytes and macrophages within the graft (105).

Immune system cells also appear to provide a variety of beneficial effects by activating astrocytes to produce angiogenic, mitogenic, chemotactic, and trophic factors that support neuronal survival and axonal regeneration (106). Microglia can provide chemoattractant stimuli for NPC and some evidence suggests microglia can bias differentiation towards a neuronal phenotype (107). Immune cells also have been implicated in contributing to the normal maintenance of adult neurogenesis in the hippocampus (108) as well as promoting tissue healing and regeneration through the phagocytic removal of dead cell debris. Thus, the role of immune system cells in CNS function and following injury is complex and efforts aimed at utilizing or disabling immune system cells of the CNS during transplantation should be done carefully while recognizing the dual effects of these cells.

4.2. Glial scar

Like other somatic tissue, the CNS attempts to heal a lesion through cellular proliferation and the deposition of ECM components. Glial scars are primarily composed of activated astrocytes that hypertrophy and secrete large amounts of chondroitin sulphate proteoglycans that inhibit axonal regrowth. Therefore the glial scar represents a chemical (proteoglycans) and physical (astrocytes) barrier to axon regeneration. Injured axons are capable of appreciable regeneration proximal to the lesion, however axons are actively inhibited as they approach the lesion epicenter and growth cones adopt a dystrophic and nodular morphology (109). The mechanism of astrocyte activation and inhibitory gliosis is still incompletely understood, however, transforming growth factor-beta (TGF-beta), and interferon-gamma (IFN-

gamma) appear to be involved. TGF-beta expression is increased immediately following CNS injury and has been shown to significantly increase proteoglycan production by astrocytes (110). IFN-gamma promotes astrocyte proliferation and IFN-gamma administration into brain lesions increases the extent of glial scarring (111). These factors may act by mediating an increase in Olig2, a transcription factor that is active during glial progenitor differentiation (112).

Chondroitinase enzyme digests most of the sugar chains on chondroitin sulphate proteolgycans and has been investigated as a means of overcoming the inhibitory effects of the glial scar (113, 114). Preventing the synthesis of sulphated proteoglycans by inhibiting the synthetic enzymes involved in glycosaminoglycan chain assembly, which are a key component of proteoglycans, can also enhance axonal regeneration (115). Removal of inhibitory molecules can aid axonal regeneration, however axonal regrowth over longer distances may require the addition of trophic factors. Neurotrophin-3 and NGF, when injected into the graft site, are capable of promoting axonal regrowth through the glial scar and into host tissue (116, 117). Thus, there exist multiple mechanisms, which can conceivably be incorporated into the design of biomaterial scaffolds, by which researchers can decrease the amount and effects of glial scarring,

5. CONCLUSIONS AND FUTURE DIRECTIONS

Biological systems operate within a multifaceted, hierarchical scale that requires the participation of specific cues orchestrated in a precise spatial and temporal fashion. This dynamic process is critical in the synthesis and regeneration of living tissue. This molecular specificity becomes more complicated due to the changing sensitivities of NPC during the process of differentiation. The microenvironment encountered by NPC transplanted to the injured brain is not conducive to their survival and integration and therefore must be manipulated to provide a more suitable interface. This can partially be accomplished via cell scaffolds that provide NPC with necessary adhesion sites that can augment cell survival. Additionally, soluble factors can be administered to bias NPC behavior and enhance the desired phenotype. These two mechanisms, suitable biomaterial and cytokine addition, are the most common methods to facilitate NPC integration. Attempts to modify the signaling environment prior to cell transplantation are less common and can involve modulating inflammation, proteoglycan activity, and host cell response.

A biomaterial-enhanced implant could, conceivably, incorporate all of the mentioned transplant techniques, although deciphering cause-effect relationships from multiple components would be formidable. Therefore, researchers have taken a systematic approach of incorporating small variations into existing transplant systems so that conclusions are unambiguous. Eventually, combinatorial approaches will need to be investigated as a means of producing more effective therapeutic strategies.

An important, yet often neglected, aspect of cell-material interactions is the molecular basis of adhesion, neurite extension, and migration. A well-designed study would describe the types of receptors and contacts that the cell uses to interact with the substrate material. This could involve cell surface receptors, such as integrins, or other adhesion molecules, such as the neural cell adhesion molecule and cadherins. This added characterization would greatly facilitate the analysis of the plethora of material substrates that have been described in the literature as "supporting" NPC growth with "potential therapeutic applications".

Although not covered in this review, understanding the intracellular mechanisms controlling NPC differentiation is the next level of knowledge required for improving NPC-based therapies. Understanding these mechanisms will greatly assist in the interpretation of results as cell behavior can then be explained on a molecular level, providing insight into the mechanisms underlying behavior of NPC.

Recently, researchers performed a detailed gene expression profiling and signaling pathway analysis of human NPC in response to a priming procedure. This priming procedure involved NPC culture on heparin and laminin surfaces, along with bFGF, a procedure that had been shown previously to increase neuronal differentiation upon transplantation (118). The research team identified sixty seven genes that had increased their expression levels two-fold, which implicated a variety of signaling pathways including the G-protein pathway, the Wnt/Notch pathway, the TGF-beta/Smad pathway, and the growth factor receptor pathway (119). This result underscores the complexity of the differentiation process and how exposure to ECM proteins can alter gene expression. Fortunately, intracellular signaling in NPC is an area of intense research. The intracellular signaling events, including transcriptional regulators mediated by specific extracellular signals, are undoubtedly responsible for the changing sensitivities of NPC during development. The ability to selectively influence intracellular protein expression in NPC via material-mediated signals should be a goal of biomaterials engineers. Precise spatial and temporal control of these signals will greatly facilitate the functional integration of transplanted NPC and ultimately provide new avenues for improved CNS therapy.

6. ACKNOWLEDGEMENTS

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Abbreviations: CNS: central nervous system, NPC: neural progenitor cells, ECM: extracellular matrix, SGZ: subgranular zone, SVZ: subventricular zone, TCPS: tissue culture polystyrene, HA: hyaluronic acid, PDL: poly-D-lysine, PGA: poly(glycolic acid), PLA: poly(lactic acid), PLGA: (poly(lactic-co-glycolic acid), NGF: nerve growth factor, PLLA: poly(L-lactic acid), PDLLA: poly(D,L-lactide), BDNF: brain-derived neurotrophic factor, poly-HEMA: poly(2-hydroxyethyl methacrylate), RGD: Arg-Gly-Asp, PHPMA: poly[N-(2-hydroxypropyl)methacrylamide], IKVAV: Ile-Lys-Val-Ala-Val, YIGSR: Tyr-Ile-Gly-Ser-Arg, PHSRN: Pro-His-Ser-Arg-Asn, BMHP: bone marrow homing peptides, TNF: tumor necrosis factor

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