Design of biomaterials to enhance stem cell survival when transplanted into the damaged central nervous system

M. J. Cooke,[a] K. Vulic[b] and M. S. Shoichet*[abcd]

Received 28th May 2010, Accepted 9th July 2010
DOI: 10.1039/c0sm00448k

Following injury to the central nervous system (CNS) there is a cascade of events that leads to cell death, tissue loss and consequently functional deficit. In response to injury, the CNS stimulates endogenous stem cell populations. However, this host repair mechanism is insufficient to restore function to the damaged tissue. Stem cells can be transplanted into damaged tissues to replace the lost cells. Although stem cell transplantation is promising, this technique is currently suboptimal. Following transplantation, significant death of the transplanted cells impedes the effectiveness of this technique. In an attempt to improve cell transplantation, groups have used scaffolds to deliver cells. In effect, biomaterials are used to create a niche that provides the appropriate microenvironment to promote survival of transplanted cells. As shown in Fig. 1, this niche includes a biomaterial with the appropriate biochemical and mechanical factors for the cells and tissues studied. In this review, we examine the mechanisms that contribute to the death of transplanted cells. We review both the *in vitro* data, where biomaterial scaffolds are designed to enhance cell survival, and the *in vivo* data, where scaffolds are shown to improve cell survival following transplantation into the damaged brain and spinal cord.

Introduction

Throughout the life of an organism cell death occurs on a daily basis in a controlled process to remove damaged or old cells. This mechanism, termed apoptosis, ensures that only the designated cells are removed from the body. During the course of normal life the rate of cell death is balanced with the production of new cells. Stem cells can divide symmetrically to replenish the stem cell pool or asymmetrically to form more specialized cells to replenish lost cells. By tightly controlling the rates of cell death and cell replacement the body can maintain cellular organization. However, upon injury to the body there is an increase in the rate of cell death due to apoptosis and necrosis. Unlike apoptosis, necrosis is not programmed and is uncontrolled. When cells die from necrosis they do not initiate the same immune response as apoptoic cells and this causes their clearance to be impeded and the release of harmful organelles, such as lysosomes, to further promote cell death.

[a]*Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 3E5, Canada*
[b]*Department of Chemistry, University of Toronto, 80 St George Street, Toronto, Ontario, M5S 3E6, Canada*
[c]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario, M5S 3G9, Canada*
[d]*The Donnelley Centre, 160 College Street, Room 514, Toronto, ON, M5S 3E1, Canada. E-mail: molly.shoichet@utoronto.ca; Fax: +1 416 978 4317; Tel: +1 416 978 1460*

M. J. Cooke

Michael J. Cooke is currently a post-doctoral fellow in Professor Shoichet's laboratory at the University of Toronto. He graduated with a BSc in Molecular Biology and Biochemistry from Durham University (UK). Cooke undertook a Wellcome Trust funded CASE PhD in the Przyborski laboratory at Durham University. His PhD was a collaborative project with Newcastle University's spin out company—Orla Protein Technologies. His PhD focused on studying how extracellular matrix proteins and motifs control stem cell fate. Currently he holds an Ontario Neurotrauma Foundation post-doctoral fellowship aimed at using biomaterials to increase cell survival following transplantation.

K. Vulic

Katarina Vulic graduated from University of Ottawa with an honours BSc in Biopharmaceutical Science. She currently holds a prestigious NSERC graduate student scholarship and is pursuing a PhD in Professor Shoichet's laboratory at the University of Toronto. Her research focuses on chemical modification of biological polymers with peptides important for cell adhesion or protein binding. These biomaterials can be used for applications in the injured central nervous system.

The central nervous system (CNS) controls the function and correct synchronisation of the body's numerous tissues. As such, injury to the CNS has significant impact on all functions of the body. Three main types of injury to the CNS are: stroke; traumatic brain injury (TBI); and spinal cord injury (SCI). Stroke defines all conditions in which the cerebral blood flow does not provide sufficient oxygen and/or glucose to the brain for an excess of 24 hours. Upon a decrease in nutrients to the brain there is a subsequent decrease in cell viability and this ultimately leads to cell death. The CNS can also become injured when an excessive external force impacts the brain or spine. No matter how the CNS is injured, the end result is cell death and loss of function.

The brain cannot repair itself following injury; however, there is evidence that endogenous stem cells are stimulated in an attempt to repair the damage.[1] In the cortex of mice, middle cerebral artery occlusion (MCAO) increases the number of nestin positive cells in the ischemic core. When explanted, these cells were demonstrated to be neural stem/progenitor cells (NSPCs) *in vitro*. Through labelling studies it was shown that these nestin positive cells originated from the cortex. When investigating the *in vivo* differentiation profile of the NSPCs in the cortex following MCAO the majority differentiated to astrocytes with some remaining nestin positive and no expression of the neuronal markers, MAP2 or Tuj1, was observed.[2] These results show that NSPCs are present in the ischemic core following a stroke, however, their differentiation is limited and mainly directed towards astrocytes. There is a similar stimulation of endogenous stem cells following injury to the spinal cord.[3]

Although the body attempts to repair itself, the stimulation of endogenous cells is limited and does not allow for functional recovery. To enhance the stimulation of endogenous stem cells, drugs have been administered and some functional recovery has been achieved.[4] Although the stimulation of endogenous cells resulted in some functional recovery, the cells produced were not organized into the correct layers within the brain and were not differentiated into the correct cell types. Furthermore, endogenous stem cell stimulation after injury decreases with age.[5] Therefore, stimulation of endogenous stem cells may not be possible in old age. An alternative strategy to stimulation of endogenous stem cells is the transplantation of exogenous cells. Transplantation of exogenous stem cells is an attractive strategy because exogenous stem cells can be produced in sufficient numbers and their differentiation is not limited.

Stem cells have the capacity to migrate from the transplantation site to the site of injury. While mesenchymal stem cells (MSCs) transplanted into uninjured mice show very little migration from the transplantation site, MSCs transplanted into an injured mouse migrate from the transplantation site to the boundary zone of the injured cortex.[6] Although cells migrate to the site of injury, it has been shown that not all cells become localized at the site of injury. When MSCs were administered into the carotid artery 2 hours following stroke, and observed 12 hours following cell injection, 95% of the cells were found in the spleen.[7] To localise transplanted stem cells to the site of injury an injection strategy where the cells are injected in close proximity to the injury site is favoured.

Although stem cell transplantation appears promising, when cells are transplanted into a lesion site they are subjected to a hostile environment and undergo cell death *via* multiple mechanisms. This leads to low levels of cell survival, 0.2–10% survival.[8–10] The reported levels of cell survival vary between groups even for similar strategies. The differences reported can be attributed to: variations in transplantation technique; cell types used (including variations in primary cell isolation); injury model; and type of animals used. For stem cell transplantation to be successful it is essential that stem cell survival is maintained. Numerous groups that have found increased cell survival correspond to increased functional recovery.[11–13] Thus to be efficacious, transplanted stem cells must survive, differentiate and integrate with the host tissue.

Mechanisms of cell death

Cell death during transplantation occurs as a result of either extrinsic factors in cell culture prior to transplantation or intrinsic factors found in the *in vivo* injury site. One day after transplantation there is a greater level of cell survival when cells are transplanted into sham injured animals (*i.e.* anaesthesia and craniotomy, but no brain injury) compared to injured animals.[9] This shows that factors in the injury site induce death of the transplanted cells. Many factors contribute to cell death following cell transplantation including: time after injury;[10,14,15] distance from the transplantation site to the epicenter of injury;[10] state of the cells transplanted—differentiated or undifferentiated;[16] developmental state of cells transplanted—embryonic *versus* adult;[17] mode of cells delivered—single cells *vs.* neurospheres;[14] host immune response;[18] and phagocytocytic response of host.[19]

The mechanisms of cell death following transplantation were investigated by Hill *et al.* in a rat T10 spinal cord level sub-acute contusion model of SCI[20] with Schwann cells (SCs) transplanted in media one week after contusion injury. Twenty two percent of transplanted SCs survived the 4 week study. Interestingly, the

M. S. Shoichet

Molly S. Shoichet is currently a Professor of Chemical Engineering and Applied Chemistry, Chemistry and Biomaterials and Biomedical Engineering at the University of Toronto. Shoichet earned an SB in Chemistry at the Massachusetts Institute of Technology and a PhD in Polymer Science and Engineering from the University of Massachusetts, Amherst. After spending 3 years as a Scientist at CytoTherapeutics Inc, she joined the faculty at the University of Toronto in 1995. Shoichet has won numerous prestigious awards including the Natural Sciences and Engineering Research Council Steacie Fellowship in 2003 and the Canada Council for the Arts, Killam Research Fellowship in 2008. She became a Fellow of the Royal Society of Canada in 2008, the Canadian Academy of Sciences. Her research expertise is in designing polymers for applications in medicine and specifically in the central nervous system and cancer.

percent of surviving cells was found to be consistent irrespective of the number of cells injected. Apoptosis and necrosis were investigated as mechanisms of cell death. Necrosis was the leading cause of death for transplanted cells during the first 24 hours after transplantation, resulting in 6 times more cell death than apoptosis. Since apoptotic SCs diminished after the first 24 hours following transplantation, the authors postulated that apoptosis may have been initiated prior to transplantation in response to removal of serum, mitogens or extracellular matrix.

During cell culture prior to transplantation, there are two main mechanisms that contribute to cell death: detachment of cells from their adherent surface and the removal of optimal growth factor concentrations. Cell death due to inhibition of cell adhesion was first reported in 1994 by Frisch and Francis.[21] Epithelial cells seeded in conditions which prevent attachment resulted in increased apoptosis. This was further confirmed by the addition of soluble GRGDSP, which prevented attachment by blocking integrins, resulting in increased apoptosis. This cell death due to a lack of attachment was termed anoikis (Greek, meaning the state of being without a home). For example, cortical neurons are unable to survive in agarose gels past 14 days of culture, but when cultured in collagen, a component of the natural ECM, cell survival was approximately 50%.[22] Anoikis can be rescued by culturing cells on ECM coated surfaces. Oligodendrocyte progenitor cells cultured on glass coverslips coated with the natural ECM proteins, fibronectin or laminin, have greater viability compared to cells cultured on PDL coated surfaces.[23] Addition of soluble laminin to NSPCs *in vitro* increases the number of neurospheres formed in comparison to control levels and reduces cell death. Blocking the β1 integrin inhibited the survival effect of laminin, suggesting a mechanism of action.[24] Although the mechanism by which anoikis occurs is not fully understood, one proposed mechanism is that following detachment, integrin stabilization of the cytoskeleton *via* plectin is lost, resulting in Bmf being released from actin. Bmf binds to Bcl-2 in mitochondria and neutralizes its anti-apoptotic effect, which activates caspase-8, releases it from the mitochondria and induces cell death.[25]

In addition to cell death by anoikis, the removal of growth factors (GFs) also induces apoptosis. When trophic support is removed, c-Jun amino-terminal kinase (JNK) is activated, which phosphorylates one of its downstream targets (c-Jun), which in turn induces the expression of a 'BH3-domain only' pro-apoptotic member of the Bcl-2 family (DP5/Hrk). It is proposed that DP5 activates Bax (a pro-apoptotic member of the Bcl-2 family), which causes mitochondrial damage leading to the release of cytochrome *c*. This leads to the formation of a cytochrome *c*/apoptotic protease-activating factor 1 (Apaf-1)/caspase-9 complex, which activates caspase-3 resulting in apoptosis.[26]

Therefore, when cells are prepared for transplantation as single cells, integrin–ECM interactions are lost and apoptosis is initiated. Cell survival is further limited by the additional cell death induced by the environment at the injury site. Using antibodies specific to the degradation product of caspase or calpain, the apoptotic and necrotic pathways can be investigated. Ab246 is specific to the caspase mediated α-spectrin degradation product and Ab38 is specific to an α-spectrin fragment generated by calpain-mediated proteolysis. By labelling the cells prior to transplantation, co-labelling can be used to determine the mechanism of cell death. When these cells were transplanted into sham injured animals, caspase-mediated cell death was seen. This could be due to damage caused by inserting a needle, trophic factor withdrawal, oxidative stress or a combination of all of these factors. Comparing the numbers of cells undergoing caspase- or calpain-mediated cell death has shown that in both sham and injured animals the level of caspase-mediated cell death is greater. In injured animals, cells undergoing caspase-mediated cell death peak at 3 days, whereas cells undergoing calpain-mediated cell death peak at 1 week post-transplantation.[9] It is clear that both apoptosis and necrosis significantly decrease cell survival.

In vitro testing of biomaterials to improve cell survival

To increase survival, cells have been delivered in biomaterial scaffolds that are designed to provide the cells with a permissive microenvironment. This microenvironment includes chemical and physical cues designed to guide cell growth and integration with the host tissue.[27] For example, the scaffold is often designed to have the mechanical properties and morphology that match the tissue in which the scaffold will be implanted while at the same time providing the cells with the appropriate cell-adhesion molecules and survival factors (Fig. 1). Thus the goal of transplanting cells in a biomaterial is to optimize the niche experienced by the cells to maintain cell viability and function. Hydrogels, which have physical and chemical properties similar to the natural extracellular matrix (ECM), are frequently used as cellular scaffolds. Natural materials which form hydrogels (see Table 1), such as collagen and agarose, have been investigated as cellular scaffolds.[22]

In order to identify a suitable biomaterial for cell delivery, it must be first tested for cytotoxicity. For example, Puramatrix, which is a peptide hydrogel, was found to be cytocompatible at 0.25% but cytotoxic at 1% to human fetal NSCs, demonstrating that gel concentration is as important as gel composition.[28] Importantly, the effect observed with NSCs may be different for another cell type or even the same cell type from another species, thus the biomaterial has to be designed and tested for a specific cell type and injury.

To prevent cell death due to anoikis, adhesion molecules are often added to the biomaterial. Non-ECM scaffolds are frequently modified with ECM proteins to improve cell survival. For example, laminin was chemically immobilized to methyl cellulose (MC), a non-cell adhesive biopolymer, which resulted in significantly higher survival of cortical neurons on laminin–methyl cellulose scaffolds compared to MC alone, or MC with physically adsorbed LN.[29] Furthermore, the viability of agarose encapsulated MSCs was increased by the addition of fibronectin and fibrinogen to the agarose capsule. Since MSCs are known to express the integrins αvβ3 and α5β1, the integrin binding of β1 was blocked which significantly decreased the viability of the encapsulated cells and thus confirmed that the fibronectin–agarose samples increased viability due to decreased anoikis.[30] Using peptide motifs, biomimetic surfaces can be produced to mimic the adhesive effects of ECM proteins[31–34] (Fig. 2). Multiple factors affect the biological response to surfaces presenting

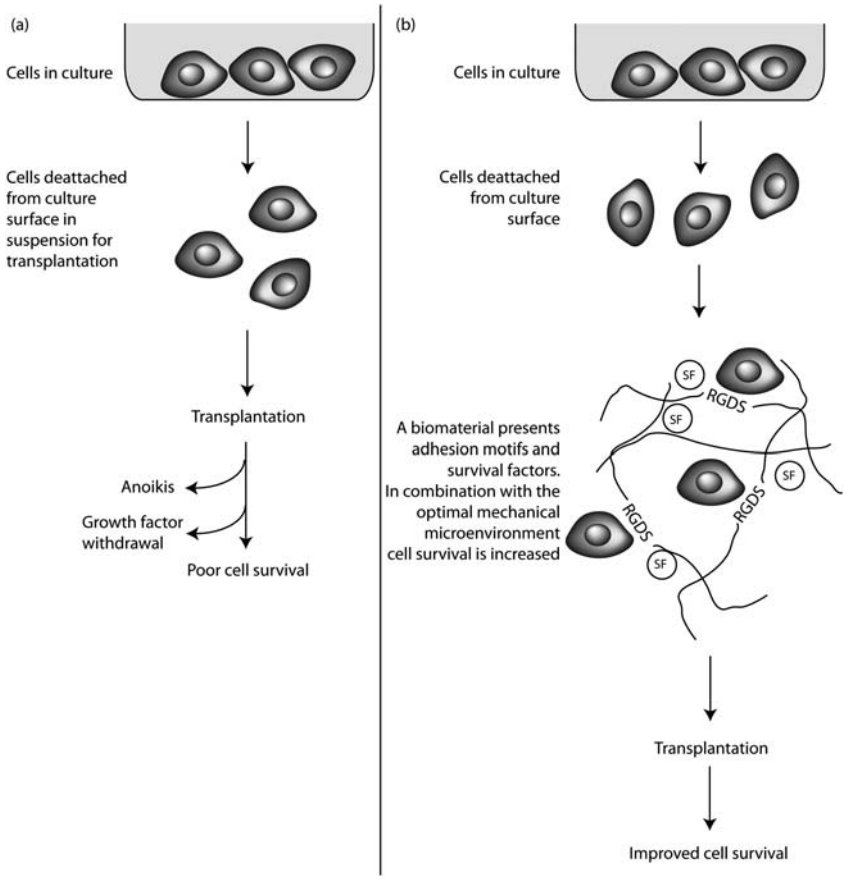

Fig. 1 Increasing cell survival using biomaterials presenting cell adhesion motifs and survival factors. (a) Cells are grown *in vitro* and prepared for transplantation by removal from their culture surface and suspension in a solution. Anoikis and growth factor withdrawal both contribute to cell death. (b) Cells are prepared for transplantation and to maintain cell viability, they are suspended in a gel or scaffold presenting cell-adhesion motifs, such as RGDS, and survival factors (SF). Furthermore, the mechanical properties of the biomaterial are tuned to further enhance cell survival.

peptides, including: cooperative effects of multiple peptides;[35] distance between the motifs presented and the surface;[36] and peptide organization.[37]

Peptides from the cell-adhesion domain of full proteins offer some advantages over full proteins because they are smaller and peptide immobilization can be better controlled in terms of the orientation and number of adhesion molecules. For example, Jongpaiboonkit *et al.*[38] found that when MSCs were cultured in a poly ethylene glycol (PEG) gel over a 7 day culture period, only 46% of cells were viable; however, cell viability increased to 81% when MSCs were cultured in IKVAV/RGDSP-modified PEG gels.

Short peptides, derived from the structures of laminin (YIGSR or IKVAV) and/or fibronectin (RGD), have been shown to promote cell adhesion to scaffolds. Non-cell adhesive dextran hydrogels were copolymerized with aminoethyl methacrylate, to provide free amines available for peptide immobilization. Hydrogels were modified with RGD or YIGSR/IKVAV cell adhesive peptides and improved the survival of DRG cells relative to unmodified dextran hydrogels.[39] A similar method was used to modify synthetic poly(2-hydroxyethyl methacrylate) (PHEMA) with IKVAV and YIGSR sequences. Peptide modified gels significantly increased the survival of dorsal root ganglion (DRG) cells after 2 days of culture.[40]

To study whether growth factors can save cells from anoikis-mediated cell death, SCs were cultured *in vitro* on a non-cell adhesive PHEMA substrate with several neurotrophic factors and then assayed to determine which GF could rescue the cells from anoikis-induced apoptosis. After 24 hours, 40% of SCs were dead and immunocytochemistry showed that a number of SCs stained positive for activated caspase-3, indicative of apoptosis. Of the factors studied, brain-derived neurotrophic factor was the only neurotrophin that successfully prevented anoikis, decreasing cell death *in vitro* to 16%. Applying this to a sub-acute compression model of SCI, to prepare cells for transplantation, the cell culture medium was supplemented with 100 ng mL^{-1} BDNF for one hour at 37 °C after detachment from the culture flask. Cells were then transplanted in SCI-injured rats (1 week after injury) and sacrificed 5 weeks later. Cell survival was significantly increased for animals which received BDNF-treated cells than controls without BDNF; however, overall cell survival for both control and BDNF-treated groups was less than 1%.[41] Thus despite the delayed delivery of cells, or co-delivery of cells with survival factors or other cell types, cell transplantation in the absence of a matrix is plagued by poor cell survival. These data support the need for cell delivery on 3-dimensional matrices, which can prevent anoikis-induced apoptosis among other cytotoxic events.

Table 1 Examples of common natural biomaterials used to promote survival of transplanted cells

Biomaterial description	Model	Effect on cell survival	Reference
Collagen and laminin-derived cell adhesive peptide	*In vitro* cell culture, embryonic rat NSCs	Increase in cell number	Hiraoka, 2009[46]
Collagen gel with diffusible NGF	*In vitro* cell culture, PC-12 cells	Increase in cell number and decrease in number of apoptotic cells	Bhang, 2009[47]
Electrospun PCL nanofibers immobilized with BDNF	*In vitro* culture, NSCs	Increase in cell number	Horne, 2009[44]
Chitosan/glycerophosphate salt hydrogels coated with PDL	*In vitro* cell culture, foetal mouse cortical cells	Increase in cell number	Crompton, 2007[49]
Elastin-like polypeptides modified with RGD	*In vitro* cell culture, PC-12 cells	Cell number comparable to collagen films	Straley, 2008[45]
P(HEMA-*co*-AEMA) modified with YIGSR and IKVAV	*In vitro* cell culture, DRG cells	Increase in cell number	Yu, 2005[40]
Dextran modified with RGDS or YIGSR and IKVAV	*In vitro* cell culture, DRG cells	Increase in cell number	Lévesque, 2006[39]
Methyl cellulose modified with laminin	*In vitro* cell culture, cortical neurons	Increase in cell viability	Stabenfeldt, 2006[29]
Laminin coated glass coverslips	*In vitro* cell culture, oligodendrocyte progenitor cells	Decrease in number of apoptotic cells	Hu, 2009[23]
Fibronectin coated glass coverslips	*In vitro* cell culture, oligodendrocyte progenitor cells	Decrease in number of apoptotic cells	Hu, 2009[23]
Agarose	*In vitro* cell culture, rat cortical neurons	Complete cell death by 14 days	O'Connor, 2001[22]
Collagen	*In vitro* cell culture, rat cortical neurons	Increase in percentage live cells over agarose	O'Connor, 2001[22]
Fibrin, Fibronectin and Fibrin/Fibronectin	*In vivo* scaffold for spinal cord knife cut lesion cavity	Increase in cell number	King, 2010[65]
Outer PLGA scaffold, inner PEG/PLL hydrogel	*In vivo* transplantation in a rat hemisection model	Increase in cell number	Rauch, 2009[70]
Chitosan channels/tubes	*In vivo* transplantation in rat complete spinal cord transection	Increase in cell number	Nomura *et al.*, 2008,[67] Zahir *et al.*, 2008[68]
Chitin/Chitosan films	*In vitro* cell culture	Increase in cell number	Freier, 2005[66]
Methyl cellulose	*In vivo* transplantation in rat spinal cord moderate thoracic (T8) contusion injury	Decrease in cell number	Patel, 2010[12]
ECM gel (laminin and collagen)	*In vivo* transplantation in rat spinal cord moderate thoracic (T8) contusion injury	Increase in cell number	Patel, 2010[12]
Fibrin	*In vivo* transplantation in rat spinal cord hemisection model	Increase in cell number	Itosaka, 2009[11]
Growth factor reduced Matrigel	*In vivo* transplantation into brain	Increased in graft size	Uemura, 2010[55]
Matrigel	*In vivo* transplantation into brain	Decrease in infarct size	Jin, 2009[56]
Matrigel	*In vivo* transplantation in rat spinal cord moderate thoracic (T8) contusion injury	Increase in cell number	Patel, 2010[12]
Ultrafoam (Collagen I)	*In vivo* transplantation into brain	Increase in cell number	Lu, 2007[57]
Hyaluronic acid hydrogel with BDNF	*In vitro* cell culture	Increase in culture viability	Nakaji-Hirabayashi, 2009[46]
Aragonite matrix	*In vitro* cell culture	Increase in cell number	Peretz, 2007[48]

Combination strategies of biomaterials and growth factors have been studied for cell delivery. It is well understood that cell survival is improved in the presence of growth factors. However, when designing a biomaterial it is important to consider how the growth factors can be co-delivered with the transplanted cells to provide a sustained and localized release. For example, nerve growth factor (NGF) was encapsulated in poly(lactic acid-*co*-glycolic acid) (PLGA) microparticles, which were then mixed with fetal rat (E16–E17) brain cells derived from the cerebral hemispheres to form a 'neo-tissue'. When compared to controls, NGF loaded microparticles increased graft function as measured by an increase in the amount of choline acetyltransferase 4, 7 and 21 days following transplantation. By attaching cells to particles with encapsulated NGF, the growth factor is released in close proximity to the transplanted cells, maximizing its effect.[42] While the addition of GFs is beneficial to cell viability, the scaffold itself must meet certain design criteria, defined by the cells and tissues of interest.

Synthetic materials such as PLGA have also promoted cell survival *in vitro* cultures of neural cells. PLGA has been investigated as it has good biocompatibility; is easily manufactured; and is believed to reduce scarring and cyst formations in models of SCI. Neural stem cells (NSCs) grafted into PLGA slices of 2 mm depth were viable after 14 days of culture.[43] Electrospun poly(ε-caprolactone) (PCL) nanofiber scaffolds promoted the *in vitro* survival of cortical cells. Similar to PLGA, PCL is biocompatible and has been investigated as a biomaterial to increase cell survival. Electrospun nanofibers can be modified to control the fiber alignment, diameter of the fibers and interfiber distance. Due to these tuneable parameters, it is proposed that

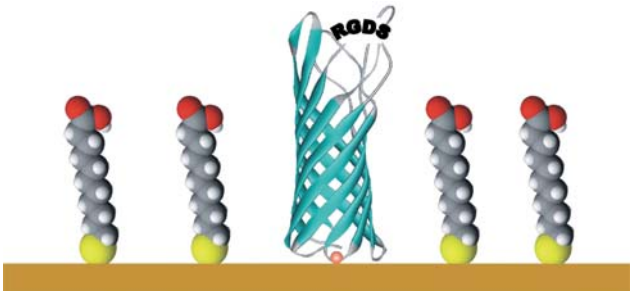

Fig. 2 The use of peptide motifs to mimic extracellular matrix proteins. The ECM protein motif RGDS is presented in a constrained loop by outer membrane protein A (OmpA) β-barrels. Spaces between β-barrels are filled using 11-mercaptoundecanoic acid by a process of self-assembly and orientation which is achieved by the terminal sulfur atom (yellow) bonding to the gold surface. From: Cooke *et al.* Neural differentiation regulated by biomimetic surfaces presenting motifs of extracellular matrix proteins.

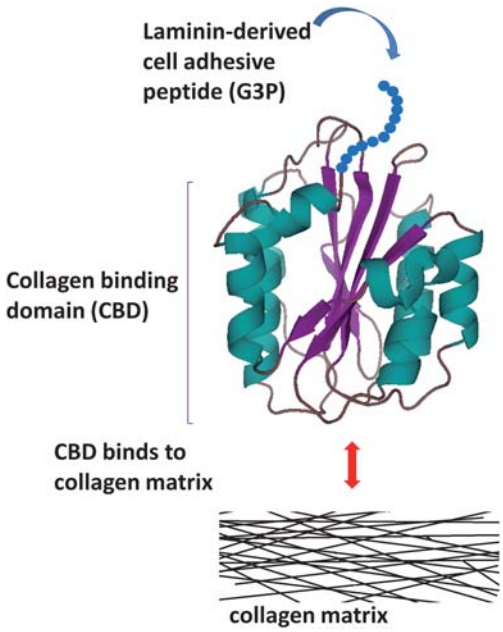

Fig. 3 Biomaterial presenting the laminin derived cell adhesion peptide G3P. The engineered fusion protein consisting of a laminin-derived cell adhesive peptide (G3P) fused to a collagen binding domain (CBD) is allowed to bind to a collagen matrix. The resultant biomaterial is a collagen matrix presenting a laminin binding peptide. Adapted from: Enhanced survival of neural cells embedded in hydrogels composed of collagen and laminin-derived adhesion peptides, Hiraoka *et al.*, 2009. Collagen binding domain from www.pdb.org.

electrospun nanofibers can provide a 3D environment to stimulate neural cells. To maintain a local supply of BDNF, PCL scaffolds were chemically modified with BDNF. Significantly greater cell survival was observed on PCL scaffolds immobilized with BDNF *vs.* PCL scaffolds with soluble BDNF or PCL scaffolds alone. However, despite increased cell survival, the proportion of apoptotic cells was not significantly reduced compared to 2D culture on PDL-coated glass coverslips.[44] While chemical modifications of scaffolds with growth factors can improve cell survival, methods to decrease cellular apoptosis on scaffolds must also be addressed.

The independent tuning of mechanical, degradation and cell adhesive properties is desirable in creating the cellular microenvironment suitable for survival. For example, scaffolds composed of alternating elastin-like structural sequences and bioactive peptide sequences provided sites for enzyme cleavage which impacted the mechanical and degradation properties while also providing sites for cell adhesion. Protease cleavage sequences sensitive to urokinase plasminogen activator (uPA), an enzyme secreted from the tips of neural growth cones, were included to allow for cell-responsive remodelling of the scaffold and RGD sequences were incorporated to improve cellular adhesion. Cell survival on RGD modified scaffolds was comparable to collagen hydrogels after 6 days of culture, and statistically greater than the control which was comprised of the identical protein polymer with a scrambled RDG sequence.[45]

Biomimetic scaffolds composed of engineered proteins facilitate the inclusion of specific binding domains while at the same time providing desired structural properties. Engineered proteins present a significant advantage as the properties of two different proteins can be combined into one protein. For example, one protein that binds to a matrix can be fused to a protein that promotes cell adhesion. This allows for the immobilization of cell attachment domains to a matrix. Using recombinant DNA technology a fusion protein termed G3P–CBD that combines a collagen binding domain (CBD) of von Willebrand factor with a cell adhesive peptide of laminin-5 (G3P) has been produced. This allows for the immobilization of a cell adhesion motif to a collagen matrix (Fig. 3). Only 21% of embryonic rat NSCs survived 2 days in culture within pure collagen hydrogels whereas significantly more cells (48%) survived on G3P-CBD collagen hydrogels. Since cell viability is largely reflective of cell adhesion to the scaffold, the authors attributed the lower cell viability of the pure collagen scaffold to anoikis. Cells cultured on G3P-CBD modified scaffolds activated anti-apoptotic cascades through binding of the α3β1 integrin, whereas without this important cell–scaffold interaction apoptotic cascades were activated.[46]

Poly(D-lysine) (PDL) is known to attract neurons and promote neurite outgrowth, and for this reason it has been used in numerous cell culture experiments. While the interaction with neurons is non-specific, PDL provides generically cell-adhesive substrates. In one study, PDL was immobilized to chitosan/glycerophosphate (chitosan/GP) hydrogels and compared to chitosan/GP alone *vs.* PDL-coated glass coverslips. PDL was modified with an azido group (4-azidoaniline) and then photochemically bound to chitosan with UV irradiation. Interestingly, on two dimensional films, 1% PDL–chitosan/GP increased survival of cortical neurons relative to chitosan/GP alone; however, there were fewer cells with neurites. This was attributed to the presence of PDL, which carries a high positive charge that may cause cell membrane damage. However, in three dimensional cortical cell cultures, 0.1% PDL–chitosan/GP hydrogels increased cell survival relative to chitosan/GP alone and increased the number of cells with neurites, but not significantly. This system provides an excellent *in vitro* substrate for cortical cell survival when immobilized with low concentrations of PDL and elucidates the importance of the concentration of cell-adhesive substrate for optimal cellular response.[49]

Improvement of cell survival *in vivo*

Cell survival can be modulated by the transplantation strategy. When NSPCs are transplanted in the compression-injured spinal cord, cell survival is <0.2% when delivered to the epicentre, yet increases to 1% when cells are injected into the spinal cord tissue rostral and caudal to the injury site. This increased survival, while modest at 7 days after implantation, decreased to 0.3% at 14 days. Notwithstanding this low survival, delayed rostral/caudal transplantation of cells to 9 days after spinal cord injury, resulted in increased cell survival to 6.5% at 7 days, but was only 2% at 14 days, after transplantation.[50]

Transplantation of alternative cell types has been proposed as a method to increase cell survival. Delivery of NSPCs or bone marrow-derived mesenchymal stromal cells (BMSCs) has been examined. Nine days after a 35 g clip compression injury, cell transplantation and survival were investigated. At 14 weeks after injection, NSPC survival was <5% and most of the surviving cells were located next to healthier tissue, at the periphery of the lesion site. Increased survival of MSCs compared to NSPCs was evident. It was estimated that up to 40% of transplanted MSCs survived to 14 weeks in some rats and had migrated towards and throughout the lesion site. Regardless of this marked improvement in survival, no functional benefit over sham controls was observed in these studies. The MSCs did not differentiate into neural cells[51] and did not integrate into the host tissue. Thus, while cell survival was increased, there was no functional benefit.

An alternative strategy is to first deliver one cell type to the site of injury to render the host environment less hostile to transplanted cells and then to subsequently transplant the cell type of interest. For example, delivery of MSCs immediately after injury can be used to improve cell survival when NSPCs are transplanted 9 days later. Cell survival in animals treated with BMSCs then NSPCs showed a non-significant increase in cell survival of 2.2% compared to 1.2% cell survival for animals receiving only NSPCs. However, significant functional improvement was observed in animals that received BMSC + NSPC treatment over those receiving BMSCs or NSPCs alone.[13]

Immune rejection decreases cell survival after transplantation. To minimize immune rejection of transplanted cells, the immunosuppressant cyclosporine was co-delivered with SCs[18] or NSPCs.[49] Co-delivery of 10 mg kg^{-1} and 20 mg kg^{-1} cyclosporine, respectively, enhanced cell survival in both cases; however, prolonged immunosuppression is problematic for the patient's overall health and thus this strategy is limited.

Improvement of cell survival using biomaterials for cell transplantation into the brain

An additional consideration when using biomaterials *in vivo* is the shape of the biomaterial. *In vivo* injuries result in cavities of varying size and shape and subsequently biomaterials that can adapt to these complex shapes are desirable. Furthermore, injectable biomaterials confer an additional advantage as they offer an easy method of delivery.[52–54] Using the knowledge gained from *in vitro* experiments, biomaterials can be designed to support the survival of transplanted cells, yet the impact of the biomaterial itself on the host tissue must be considered. Several groups have demonstrated that cell transplantation within biomaterials is better than controls without biomaterials in terms of cell survival and behavioural recovery.[55–57] For example, in the delivery of retinal stem cells to the retina, Ballios *et al.*[53] found that cell survival was significantly improved when the cells were delivered in an injectable hydrogel of hyaluronan and methyl cellulose than when injected in conventional saline. Here the biomaterial limited cell aggregation and promoted cell distribution, which enhanced survival and host tissue integration (Fig. 4). In another example, MSCs co-injected into the brain of TBI rats with an Ultrafoam collagen I scaffold, promoted greater tissue healing than transplantation of MSCs in saline. Upon histological examination of brains co-transplanted with cells and a collagen I scaffold there was a significant decrease in the lesion volume compared to cells transplanted in saline.[57]

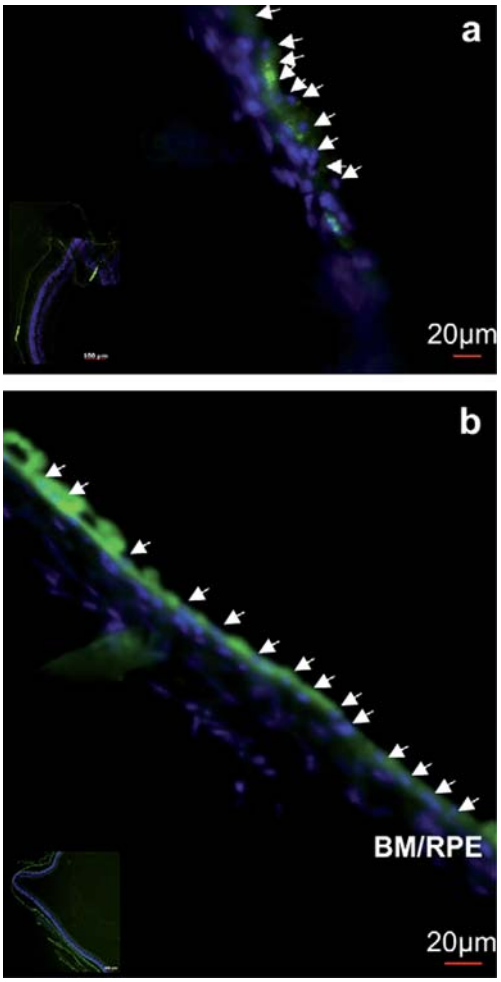

Fig. 4 The biomaterial HAMC improves integration of retinal stem cells. Cells transplanted in aCSF (a) showed non-contiguous integration compared to those transplanted in HAMC (b). From: Ballios *et al.* A hydrogel-based stem cell delivery system to treat retinal degenerative diseases.

Biomaterials have been investigated alone to promote healing. For example, when a hydrogel blend of hyaluronan and methyl cellulose (HAMC) was injected across the dura into the intrathecal space surrounding the spinal cord, the dura was observed to heal within 4 weeks whereas control animals that had an artificial cerebrospinal fluid (aCSF) injected were left with a torn

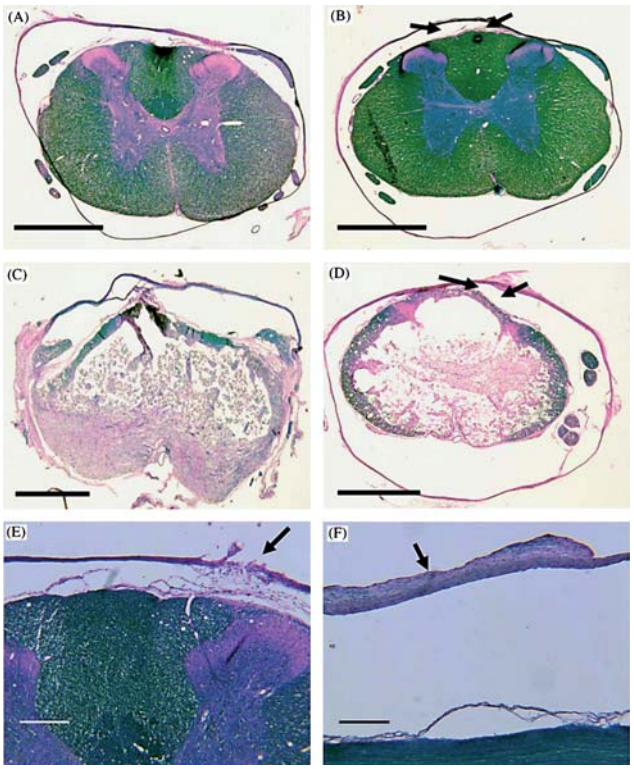

Fig. 5 The biomaterial HAMC has a beneficial effect on healing when compared to control animals. Uninjured rat injected with aCSF (A), or HAMC (B). Spinal cord injured rat injected with aCSF (C), or HAMC (D). Injection of aCSF into uninjured animals left a gap in the dura (E) whereas, HAMC had a beneficial effect on dura integrity (F). Staining: Luxol Fast Blue and counterstained with hematoxylin and eosin. Scale bars: A–D 1 mm; E and F 200 μm. From: Gupta et al. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord.

dura[54] (Fig. 5). Interestingly, the inflammatory response in the spinal cord tissue was consistent with dura healing; there was a reduced inflammatory response in the spinal cord tissue of those animals that received HAMC *vs.* aCSF. Hyaluronan has been shown to promote wound healing in many tissues, although the mechanism is not fully understood.[58,59] Similarly, beneficial effects of other biomaterials have been reported. For example, hyaluronic acid hydrogels alone, without any cells, have beneficial effects on healing of injured brain tissue such as decreasing the glial scar and increasing angiogenesis.[60]

Both natural and synthetic biomaterials, described above, have been investigated for stem cell transplantation. Matrigel, which has been studied for cell delivery, is an extracellular matrix extract from mouse sarcoma comprised of ECM proteins, such as collagen and laminin, and several growth factors including fibroblast growth factor 2 (FGF-2) and epidermal growth factor (EGF). To test whether Matrigel could increase cell survival after transplantation, neural progenitor cells (NPCs) were injected three days after MCAO either in Matrigel or after culturing in Matrigel *vs.* transplanted in aCSF. The infarct volume decreased significantly when filled with cells cultured or injected with Matrigel *vs.* those injected in aCSF. While few of the injected human NPCs injected with aCSF could be detected in the vicinity of the infarct 8 weeks following transplantation, cells transplanted with Matrigel had numerous cells present at the infarct site while transplantation of cells cultured in Matrigel had the most abundant number of cells at the infarct site.[56] Notwithstanding these interesting results, Matrigel is derived from a mouse sarcoma and is thus an ill-defined matrix unsuitable for translational science.

The individual ECM proteins of Matrigel have been investigated themselves as scaffolds for transplantation. For example, collagen I is a fibrous protein composed of three α subunits wound in a triple helical structure forming a rigid superhelix and has been used to deliver MSCs into traumatic brain injured rats. MSCs transplanted alone in saline showed beneficial effects and reduced behavioural deficits; however, MSCs transplanted in a collagen I scaffold reduced the behavioural deficits even further, thereby demonstrating a benefit of the biomaterial delivery vehicle. In order to understand whether it was the biomaterial itself or the impact of the biomaterial on the cells delivered that resulted in the improved functional outcome, the number of MSCs at the lesion boundary site were investigated. Cells transplanted in the collagen I scaffold were able to migrate into the lesion boundary and were significantly more abundant than hMSCs transplanted in saline[57] suggesting that the beneficial effect of collagen delivery was cell survival.

In another cell delivery strategy to TBI rats, MSCs were seeded into a collagen I gel and cultured overnight prior to transplantation. Those animals that had MSCs transplanted in collagen I scaffolds (*vs.* those that had MSCs transplanted without collagen I) showed significant improvements in the modified neurological severity score and Morris water maze test. Interestingly, cells transplanted in a collagen I scaffold showed greater angiogenesis compared to cells transplanted without a scaffold.[61]

Building on the importance of the ECM to cell survival, Tate *et al.* investigated composite gels of collagen I with either adsorbed laminin (LN) or fibronectin (FN) in terms of cell survival in a cortical impact injury model in rats. Foetal NSCs, isolated from the germinal layer, showed only a 2–3% survival in all groups tested one week after transplantation in: collagen I/laminin, collagen I/fibronectin, or media. Interestingly, 8 weeks post-transplantation, the percent survival was still low; however, differences in the number of surviving cells were evident with significantly greater number of cells transplanted in the collagen I/LN *vs.* those in collagen I/FN and media alone, which had the fewest surviving cells. The cells were found to be negative for phospho–histone H3, indicating that the cells were not actively proliferating and thus the observations were due to cell survival and not cell proliferation.[62]

Synthetic biomaterials have better-defined chemical structures and origins than naturally derived materials which can be advantageous. Polyglycolic acid (PGA) was investigated as a scaffold for NSC transplantation into the injured brain. NSCs were transplanted alone or on PGA scaffolds (on which they had been cultured in PGA for 4 days) into the brain 7 days post-injury. The injury-induced cavity filled with new parenchyma and there was minimal monocyte infiltration into the NSC–PGA complex at the interface between the complex and the host cortical penumbra whereas there was significant monocyte infiltration in the untransplanted infarct controls. Astroglial scarring

was also reduced in PGA–NSC transplanted groups relative to non-transplanted infarcts. It was proposed that the reduction in astroglial scarring was due to either inhibitory factors produced by the NSCs, the mechanical features of the scaffold or the actions of the NSC–scaffold complex upon the host's injury response.[63]

To support NSC survival following transplantation into the brain, NSCs have been delivered on fibronectin-coated PLGA particles which provide sites for cell adhesion.[64] This combination strategy was further advanced by Mahoney and Saltzman[42] who used PLGA microparticles, 0.5–5 μm in diameter, encapsulating NGF to deliver fetal brain cells. Neo-tissues, comprised of fetal brain cells and NGF eluting PLGA particles, were transplanted into rat caudate/putamen where NGF release was observed for up to 4 days and the ChAT activity was significantly greater for the 21 days tested than controls comprised of fetal brain cells cultured on PLGA microspheres eluting BSA. Thus the composite NGF eluting PLGA microspheres resulted in greater cell viability than controls eluting BSA.

Improvement of cell survival using biomaterials for transplantation into the spinal cord

Though many natural and synthetic polymers have been investigated *in vitro* as cellular scaffolds to promote cell survival, few of these scaffolds have been utilized in *in vivo* cell transplantation applications for SCI. Fibrin is one of these exceptions and it has been used to transplant GFP-positive BMSCs into the cavity formed after a hemisection spinal cord injury model in the rat. After 4 weeks, animals were sacrificed and histological analysis showed that no GFP-positive cells persisted when MSCs were delivered in media suspension. However, when delivered in a fibrin scaffold, numerous GFP-positive cells were detected. Increased cell survival also resulted in a significant functional improvement for animals that received MSC + fibrin *vs.* just MSCs alone, or a vehicle control of PBS. Interactions between MSCs and the fibrin matrix allowed for MSC migration and adherence, preventing anoikis, which improved their survival after transplantation.[11] In another study, collagen, fibrin (FIN), fibrinogen (FB) or a mixture of fibrin/fibrinogen (FIN/FB) scaffolds were compared in terms of endogenous cell survival after implantation into a lesion cavity in rats. At one week after implantation, collagen implants comprised very dense areas which contained large cavities and thus these samples were not further evaluated. At four weeks after implantation, cell survival around FB and FIN/FB gels was statistically greater than survival of cells around FIN alone. Fibrin and FIN/FB gels showed good integration with host tissue whereas collagen and FIN scaffolds contained dense cavities, especially at the material–host interface. These data demonstrate the importance of the biomaterial in terms of promoting endogenous cell survival.[65] The utility of methyl cellulose (MC), Matrigel, or an ECM gel, composed of laminin and collagen IV (CN/LN) as delivery vehicles for transplanted SCs was examined in a moderate contusion injury model in rats. Twelve weeks post-implantation, 14% of transplanted SCs survived when delivered in media suspension. Cells transplanted in the CN/LN scaffold showed a significant increase in survival with 27% of transplanted cells surviving. Matrigel scaffolds resulted in even greater cell survival with 36% overall survival. In contrast, MC was unable to support cell survival, significantly reducing cell survival to 2%. Poor cell survival of SCs transplanted within MC hydrogels reflects the non-cell adhesive nature of MC where important cell–scaffold contacts are inhibited. Cell proliferation accounted for approximately 3–5% of implanted SCs. A strong correlation between the degree of SC survival and the vascularisation of the injury-implant site was observed. The increased angiogenesis of CN/LN and Matrigel scaffolds resulted in a greater supply of nutrients and oxygen to transplanted cells, which improved their survival. Methyl cellulose scaffolds had poor angiogenesis and the lowest SC survival.[12]

Non-ECM derived natural materials have also been used in cell transplantation strategies to improve cell survival. Chitosan and chitin films were shown to promote cell survival *in vitro*[66] and investigated as cell guidance channels to promote survival of transplanted NSPCs. Three million brain-derived or spinal cord-derived NSPCs were seeded in chitosan tubes coated with laminin and implanted in the injured rat spinal cord after transection of the cord. Brain-derived NSPCs showed a significantly greater survival than spinal cord-derived NSPCs 12 weeks after transplantation, yet the increased cell survival did not translate to improved functional recovery or axonal regeneration.[67,68] In a combinatorial approach, endothelial cells (ECs) and NSPCs were co-delivered in a two-component biomaterial composed of an outer PLGA scaffold and an inner poly(ethylene glycol)/poly-L-lysine (PEG/PLL) macroporous hydrogel to the injured rat spinal cord in a hemisection model of SCI. Endothelial cells were included to promote vascularisation within the transplant to increase cell survival. At eight weeks post-transplantation, the number of functional blood vessels at the lesion site for NSPC/EC + implant animals was significantly greater compared to the NSPC + implant. Interestingly, the NSPC/EC + implant was the only group that reformed the blood–spinal cord barrier on the lesioned side of the injury epicentre. Surprisingly, increased vascularisation did not result in increased NSPC survival: at 8 weeks post-transplantation, NSPC survival was 8% in the NSPC/EC + implant group *vs.* 20% in the NSPC + implant group. The authors attributed this unexpected result to the different number of NSPCs originally transplanted. Since NSPCs produce a number of survival factors,[69] which promote cell survival and 4.5 times more NSPCs were transplanted in the NSPC + implant group than the NSPC/EC + implant group, the difference in cell survival may be attributed to the greater number of NSPCs secreting more survival factors.[70]

Conclusions

Stem cell transplantation presents a viable strategy for the repair of CNS injury. However, following transplantation cell death is prevalent and limits the efficacy of this technique. Two of the factors that contribute to poor cell survival are anoikis and growth factor withdrawal. Biomaterials can be modified with cell adhesion proteins or motifs to improve cell attachment and minimize cell death caused by anoikis. Furthermore, survival factors, such as growth factors, can be encapsulated into the biomaterial to enhance cell survival. By using biomaterials to minimize cell death and promote cell integration with host tissue,

more regenerative medicine strategies will be successfully translated to the clinic.

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