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# Biomaterials for Brain Tissue Engineering

*Jerani T. S. Pettikiriarachchi,*[A] *Clare L. Parish,*[B] *Molly S. Shoichet,*[C] *John S. Forsythe,*[A] *and David R. Nisbet*[A,D,E]

[A]Department of Materials Engineering, Monash University, Vic. 3800, Australia.
[B]Florey Neuroscience Institutes, University of Melbourne, Parkville, Vic. 3010, Australia.
[C]Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, ON M5S 3E5, Canada.
[D]The Mental Health Research Institute of Victoria, Parkville, Vic. 3010, Australia.
[E]Corresponding author. Email: david.nisbet@eng.monash.edu.au

Neurological disorders such as traumatic brain injuries or stroke result in neuronal loss and disruption of the brain parenchyma. Current treatment strategies are limited in that they can only mitigate the degeneration process or alleviate the symptoms but do not reverse the condition. In contrast, regenerative cell-based therapies offer long-term hope for many patients. Bioactive scaffolds are likely to reinforce the success of cell replacement therapies by providing a microenvironment that facilitates the survival, proliferation, differentiation, and connectivity of transplanted and/or endogenous cells. This Review outlines various biomaterials (including hydrogels, self-assembling peptides, and electrospun nanofibres) that have been investigated for the repair of brain tissue, and discusses strategies for the immobilization of biomolecules. An overview of the potential clinical applications of such scaffolds in neurodegenerative diseases is also provided.

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## Introduction

The adult brain has limited regenerative capacity.[1,2] Consequently, tissue insult resulting from disease or traumatic brain injuries (TBI) is permanent and can result in several symptoms including cognitive, motor and psychotic dysfunction. Current clinical treatment strategies focus on minimizing further tissue loss and/or alleviating symptoms through administration of pharmacological agents as well as maintaining motility through rehabilitation. However, these treatments have limited effectiveness, with some being associated with unwanted side-effects.[3] The objective of brain tissue engineering is to repair, replace, and regenerate tissue at the damaged site in order to re-establish functionality at both the cellular and organ levels.

Cell loss following neural insult disrupts the connectivity and signal transmission between neurons, adversely affecting function. Additionally, progressive degeneration typically results in the activation of astrocytes, microglia or macrophages, and oligodendrocyte precursor cells that contribute to glial scar formation, and can also be accompanied by the formation of acellular voids at the afflicted site.[4] Therefore, successful cell therapies to replace lost neurons and/or prevent further degeneration is underpinned by several mechanisms including neuroprotection, creation of cellular microenvironments for neural regeneration, expression of trophic factors, vascularization, and promotion of guided axonal outgrowth.[5] Cumulatively, these factors act to enhance cell survival and connectivity, and re-establish a functional neural network.

The creation of an artificial microenvironment to support neuron survival (endogenous or transplanted cells) as well as their integration is an essential feature, not only in terms of facilitating cell regeneration but also in enacting a form of architectural support to prevent further damage to adjacent tissue. In recent years, there has been much research dedicated towards the construction, as well as biochemical and biophysical optimization, of preformed scaffolds such as electrospun nanofibres, and injectable scaffolds such as hydrogels and self-assembling scaffolds for brain repair.

## Brain Structure and Strategies for Repair

The intricate structure of the brain comprises highly organized interconnected neurons that interact with the extracellular matrix (ECM) to form a complex network.[6] During development, neural cells proliferate and migrate into discrete locations within the brain in response to various trophic cues.[7] Neurons also transduce topographical stimuli through interaction of the growth cone (a specialized axon tip containing filopodia) with the immediate environment[8,9] and mechanical cues that can direct neurite extension.[10] Guided neurite and axonal growth ensures appropriate and regulated connectivity within the overall neural circuitry, giving rise to specialized nuclei with specific functions within the brain.

Understanding and replicating many of these developmental events will be crucial for promoting neural regeneration. For example, neurotrophins (such as glial-derived neurotrophic factor, GDNF and brain-derived neurotrophic factor, BDNF) are important in the connectivity and survival of neurons in development. In animal models of Parkinson's Disease (PD),[11–13] Huntingson's Disease,[14,15] and TBI,[16] overexpression of these trophins can promote cell survival and integration, as

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well as enhance the success of cell replacement therapy.[13,17] Additionally, mitogens and morphogens that regulate neuronal proliferation and differentiation can similarly promote the engraftment of transplanted cells in animal models of neurodegenerative diseases.

## Design Criteria: Designing Scaffolds to Promote Neural Repair

To promote neural regeneration within an unconducive environment, a scaffold needs to regulate cell adhesion, proliferation, migration and neurite elongation, recapitulating some of the events that occur during embryogenesis. Furthermore, this must occur within a three-dimensional (3D) architecture to allow for relevant and appropriate tissue reformation. In order to maintain cell functionality and encourage repair of the neural circuitry, scaffolds should facilitate fluid flow, supplying nutrients to cells while eliminating metabolite wastes. These scaffolds should also present cells with appropriate temporal and spatial molecular cues to achieve directed cell maturation and integration. Consequently, scaffolds with interconnected porosity that comprise of sufficiently large pores and appropriate surface functionality are required for cell migration. In addition, physical support must be offered to cells and axons, as well as physical properties similar to the native environment (e.g. elastic modulus). This poses a major scaffold design challenge because native brain tissue typically has an elastic modulus of 0.5–1 kPa.[18,19] Neural cells sense mechanical properties such as matrix stiffness and respond through cell colonization, migration and biased differentiation,[10,20] and altered neurite formation and trajectory,[21,22] as shown in Fig. 1. For instance, after 8 days of stem cell culture on photopolymerizable methacrylamide chitosan hydrogels with stiffness between $<1$ and 7 kPa, biased cell differentiation was observed such that the $<1$ kPa substrate produced 59% oligodendrocytes, 33% neurons, and 2% astrocytes, while the 7 kPa substrate produced 72% oligodendrocytes, 12% neurons, and no astrocytes, and the 3.5 kPa substrate yielded intermediate values.[10] Furthermore, the rate of neurite extension of dorsal root ganglion cells is inversely proportional to substrate stiffness[21] and neurons produce more primary dendrites and shorter axons on stiffer substrates.[22]

In addition to optimizing the morphological and mechanical properties of a scaffold, another approach to enhancing host

*Jerani Pettikiriarachchi completed her Bachelor of Science and Engineering double degree in 2008 at Monash University. She was awarded an Australian Postgraduate Award scholarship to pursue postgraduate studies in Biomaterials Engineering at Monash University under the supervision of Dr John Forsythe and Dr David Nisbet. In 2010, she successfully transferred from a Masters degree to Ph.D. Jerani's research is focussed on the fabrication of biologically functional nanofibrous scaffolds for spinal cord injury applications.*

*Clare Parish completed her undergraduate studies in Biomedical Science at Monash University, Australia. She obtained a Ph.D. in neuroanatomy from Monash in 2002. Dr Parish received two training fellowships (Human Frontiers Science Program long-term training fellowship and an Australian NHMRC CJ Martin fellowship) to pursue a 4-year postdoctorate in molecular and stem cell biology at the Karolinska Institute (Stockholm, Sweden). In 2007, she returned to Australia to establish an independent research career at the Florey Neurosciences Institutes in Melbourne. In 2009, Dr Parish was appointed head of the Stem Cell Therapy laboratory at the Institute and awarded a prestigious NHMRC Career Development Fellowship. Her research interest is focussed on developing and improving cell replacement therapy strategies to repair the injured brain. Dr Parish is also interested in understanding how the brain matures and is wired during development, knowledge critical for repairing neural pathways.*

*Professor Molly Shoichet graduated from MIT and completed her Ph.D. in Polymer Science and Engineering at the University of Massachusetts, Amherst, in 1992. She is currently a Professor and Canada Research Chair in the Department of Chemical Engineering and Applied Chemistry at the University of Toronto, where she researches tissue engineering strategies to promote regeneration after central nervous system injury. Her novel work has earned her many awards, including the prestigious Canadian Council for the Arts Killam Fellowship.*

*Dr Forsythe's major research interest is in the field of neural tissue engineering. His research is focussed on biochemical and biophysical cues to control cell function to ultimately repair neural pathways damaged as a consequence of Parkinson's disease and spinal cord injury.*

*David Nisbet studied engineering at Monash University, Australia, before completing his Ph.D. in 2009. He was then awarded an Australian Postdoctoral Research Fellowship. Dr Nisbet's research is aligned with new scientific directions, having expertise that bridges the traditional fields of materials engineering, nanotechnology, surface science and biology. He currently has two main research areas, which include neural and bone tissue engineering. For more information, please refer to the website http://www.eng.monash.edu.au/mat/staff/nisbetd.html*

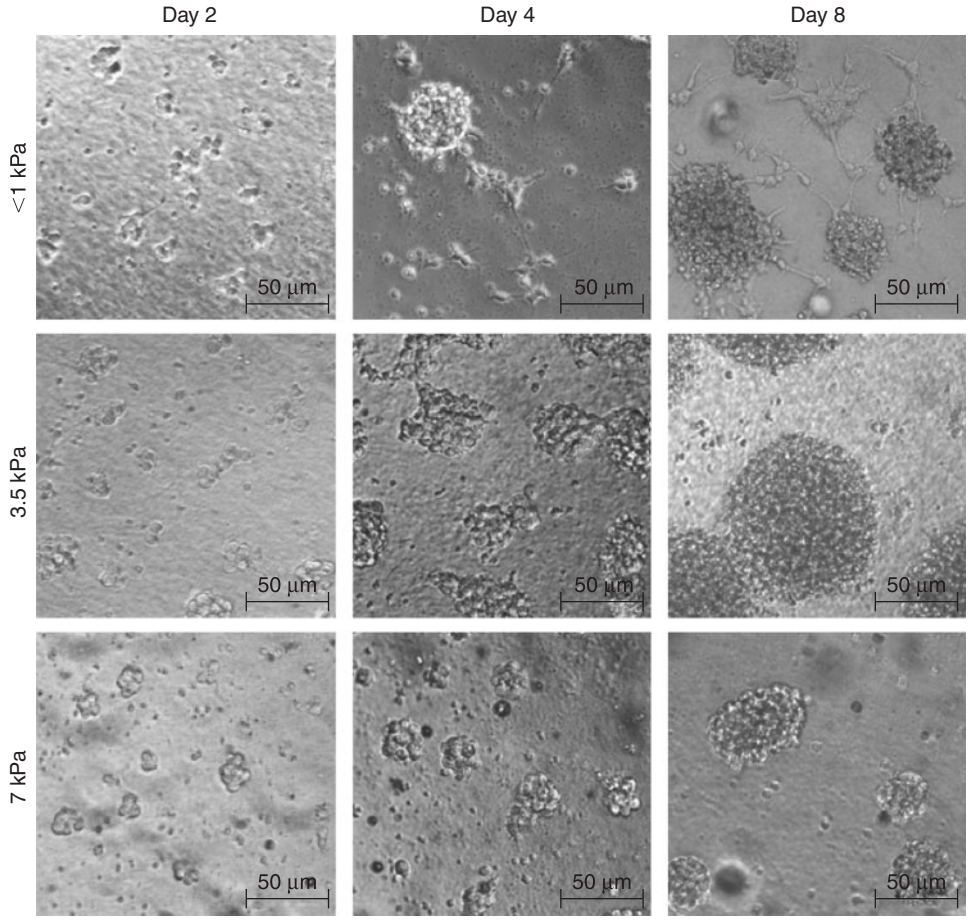


**Fig. 1.** Micrographs of neural stem or progenitor cells cultured on methacrylamide chitosan substrates of varying elastic moduli over 8 days. Single cells attached to all surfaces and proliferated over time to form colonies. Largest cell colonies occurred on the 3.5 kPa substrate while smaller colonies formed on the 7 kPa substrate. Cell migration out of colonies and neurite formation was observed only on the <1 kPa substrate. Reprinted from ref. [10], with permission from Elsevier.

tissue integration is to ensure the polymers utilized in scaffold manufacture and its degradation products are non-cytotoxic and non-inflammatory.[6,23] The concept of scaffold biodegradation has both benefits and drawbacks; however, this issue must be addressed in terms of the primary injury. Although biodegradation enhances scaffold porosity over time and allows cell infiltration, it diminishes the mechanical integrity of the scaffold and can lead to build-up of non-bioeliminable by-products in the body. Consequently, for small lesions in other parts of the body, it may be desirable to produce a biodegradable scaffold that deteriorates as cells deposit their own ECM. However, in the brain, particularly for large lesions such as those caused by TBI, it is more feasible to have a long-term scaffold providing architectural support of the adjacent brain parenchyma, while also supporting cell differentiation.[24–26]

In terms of clinical application, scaffolds should be designed to be able to be implanted in a minimally invasive manner. The employment of preformed scaffolds in brain tissue repair may present difficulties in that the implantation is a highly invasive procedure compared with injectable scaffolds. However, preformed scaffolds generally have superior manufacture tailorability and mechanical integrity.

To design a scaffold that recapitulates many of the morphological features of the brain is a challenging task given its highly specialized and organized structure. In order to attempt this, the basic scaffold features need to be further optimized before implantation within the brain. Optimization of these scaffold traits will supply cells with the factors essential for sustenance and facilitate cell permeation of the scaffold. Whether these scaffolds will carry neural stem or progenitor cells, or encourage the elongation of existing axons[27,28] and cells in the penumbra to penetrate the construct, or both, remains to be determined. Traversal of the scaffold by axonal growth of surrounding neural cells will take time; therefore, seeding scaffolds with cells may promote more rapid interconnectivity. However, seeding of heterologous and homologous neural progenitor cells will elicit host immune-system reactions, resulting in implant rejection unless immune system suppressants are also prescribed to patients. The implantation of a preformed scaffold containing neural progenitor cells into the injured mouse brain demonstrated a capacity for host- and donor-derived neurons to form a meshwork and reconstitute some anatomical connections while reducing inflammation and scarring.[29] It is the authors' opinion that ultimately the scaffold will require seeding of autologous or homologous neural cells to facilitate the repair process.

## Scaffolds for Brain Repair

A range of scaffolds including hydrogels, self-assembling peptides, and electrospun nanofibre scaffolds have been investigated

as candidates for neural tissue engineering within the brain. Each scaffold is manufactured via distinct techniques and therefore they exhibit variations in their morphology. As well as considering the mechanical properties of the scaffold for brain tissue engineering, it is essential that the surface properties are optimized to support endogenous or implanted cells and to possibly provide guided axonal growth. The trade-offs in bulk and surface properties may necessitate optimization of the scaffold through means such as incorporating biomolecules and surface treatment procedures for improved biorecognition. The subsequent sections will review the various scaffolds and outline methods of modification employed to enhance neural integration and regeneration following implantation into the brain.

## Hydrogels

Hydrogels are hydrophilic polymer networks that can absorb ~30% (as a lower limit) of their dried weight in water.[30] Dissolution of the polymer network in water is hindered through the formation of crosslinks, which can be classified as physical or chemical. Physical crosslinks rely on chain entanglements and secondary forces whereas chemical crosslinks are formed via covalent bonds.[31,32] The network morphology of isotropic hydrogels gives rise to small mesh-like structures in which the limited spacing between crosslinks prevents cell migration. However, hydrogels can exhibit either micro- or macroporosity, the latter of which is typically employed in tissue-engineering applications owing to the relatively large pore sizes (10–100 μm in diameter)[33] that allow cell and axon infiltration. The mesh structure and highly interconnected porosity of hydrogels accounts for the high water content and enables rapid diffusion of nutrients and metabolites to and from the cells.[34] Although these features make hydrogels compatible with surrounding tissue, they adversely affect the mechanical integrity of the scaffold, rendering it susceptible to collapse in vivo.

An advantage of hydrogels is that their mechanical properties can be tuned to be similar to that of soft tissue such as the brain. This can facilitate the transfer of mechanical stimuli to cells, which parallels that of native tissue. Generally, the mechanical properties of hydrogels are tuned through regulation of the crosslink density.[35] Furthermore, some hydrogels exhibit a composition-dependent critical temperature (Lower Critical Solution Temperature (LCST)[36,37] or Upper Critical Solution Temperature (UCST))[38] at which gelation or phase separation occurs. Thermoresponsive gelation serves several functions such as facilitating injection of the scaffold into a lesion via a minimally invasive procedure, while also enabling the hydrogel to interface with irregular cavities. Such hydrogels are also an asset in cell replacement therapy as they provide a controllable, 3D microenvironment for the proliferation and differentiation of stem cells, while their thermoresponsive nature can facilitate cell encapsulation.[39,40]

### *Biologically Derived Hydrogels*

Biologically derived (natural) polymers have enhanced biocompatibility due to similarities with polymers found within the body.[41] Most biologically derived hydrogels are polysaccharides and glycosaminoglycans, some of which are constituents of the ECM such as hyaluronic acid (HA). Natural polymers can possess inherent bioactivity, eliminating the necessity for biomolecule functionalization to achieve cell–scaffold interactions. However, owing to their biological origins, natural polymers are also potentially susceptible to biodegradation via enzymatic action, which can be beneficial in promoting cell and neurite penetration into the hydrogel[42] but may also prematurely compromise the mechanical integrity.

The most common natural polymers in neural tissue engineering are collagen[43–46] and HA.[40,47–49] Although collagen does not naturally occur in the brain, it has been shown to support neural cell attachment and proliferation.[23,50,51] Collagen scaffolds infused with nerve growth factor (a neurotrophin that rescues and protects cells in dying tissue) are capable of improving cell viability in vitro.[43,44] Furthermore, neurons cultured in collagen hydrogels retained their capacity to generate spontaneous post-synaptic potentials, demonstrating functional synapse formation.[46] However, it is also important to determine whether these electrophysiological properties coincide with what occurs in vivo in terms of magnitude and frequency. Interestingly, implantation of a collagen scaffold embedded with human marrow stromal cells (hMSCs) into the lesioned rat cortex was capable of improving spatial learning, sensory-motor function and cell infiltration, and reduced lesion volume.[45] This demonstrates the potential of collagen scaffolds as cell-delivery platforms in the treatment of TBI, where as a result of enhanced cell anchorage and support structure, they improve cell survival and migration of hMSCs to the lesion boundary zone to promote repair.

HA is a high-molecular weight glycosaminoglycan and is a constituent of the brain ECM.[52] HA hydrogels have been chemically and physically modified with polylysine, homopolypeptides, and anti-NgR (an inhibitor of the Nogo complex myelin-associated proteins)[40,47] to further the regenerative capacity of the brain. These treatments improved neural progenitor cell attachment[40] and promoted neuronal-like morphology in primary hippocampal cells.[47] However, polylysine modification appears to have ambiguous effects, possibly due to different concentration used,[37,53] with separate studies reporting promotion[40] and conversely inhibition[48] of neural differentiation. However, HA scaffolds with polylysine and anti-NgR immobilized synergistically enhanced neural cell proliferation by approximately six-fold compared with HA, and two-fold compared with both HA with anti-NgR and HA with polylysine.[47] Implantation of HA scaffolds immobilized with argenine–glysine–aspartate (RGD) peptides into rat cortex lesions supported cell infiltration, angiogenesis, neurite extension, and minimized glial scarring.[49] A scanning electron micrograph image of hydrogel integration with host tissue is depicted in Fig. 2. Similarly, a 1:20 HA–gelatin (irreversibly hydrolyzed form of collagen) blend scaffold and a gelatin scaffold implanted into brain tissue and analysis during a 4- to 13-week period exhibited good compatibility, with the blend scaffold exhibiting better congruity.[54]

Other natural polymers used in neural tissue engineering include fibrin, methylcellulose, chitosan, and alginate. Fibrin is a fibrillar protein derived from fibrinogen that functions as a bridging molecule for cell–cell interactions and binds to cell-surface receptors at injury sites to promote clotting.[55] Implantation of fibrin gels in the spinal cord improved the survival and migration of transplanted bone marrow cells and neural recovery compared with cell therapy alone,[56] and furthermore delayed reactive astrocyte recruitment and enhanced neuronal migration.[57] Although implanted in the spinal cord, it is believed that implantation of fibrin gels in the brain would yield similar results.[56] Methylcellulose is synthesized from cellulose via a substitution reaction of hydroxyl groups with methoxide. Gelation of methylcellulose is a temperature-dependent process

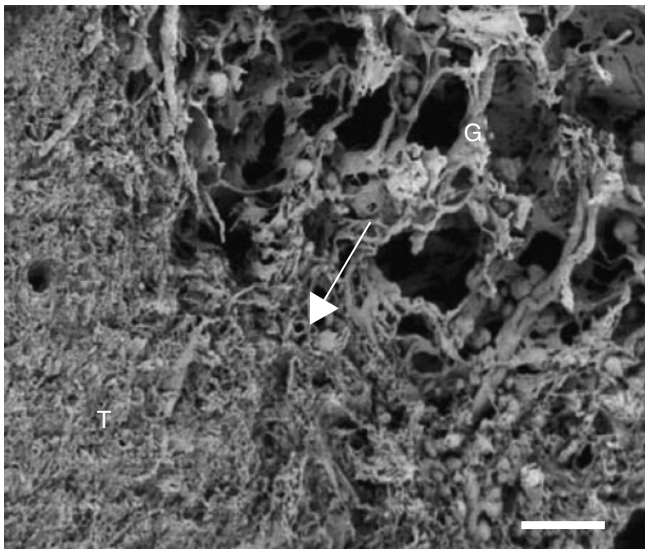


**Fig. 2.** Scanning electron micrograph image of a hyaluronic acid hydrogel in the rat brain 6 weeks after implantation. The arrow indicates the interface between hydrogel and tissue. Scale bar = 40 μm; G is the hydrogel implant and T is the host tissue. Reprinted from ref. [49], with permission from Springer Science + Business Media.

where temperatures at or above 60°C produce a phase-separated gel; however, the gelation point can be altered by changes in composition.[58,59] The bioactivity of methylcellulose was increased through conjugation with laminin, as demonstrated through enhanced cortical neural cell adhesion.[36] Neuronal cell attachment was dramatically increased in excess of 15-fold in chitosan–agarose blended hydrogels compared with agarose, owing to non-specific electrostatic interactions between chitosan and the cell membrane. In addition, hydrogels with higher agarose concentration promoted linear expression of neurites whereas those with higher chitosan concentrations expressed tortuous paths with greater branching.[60] However, agarose–chitosan hydrogels only form a homogeneous phase under acidic conditions (owing to the electrostatic effect among protonated amine groups in chitosan) and undergo phase separation due to deprotonation of amine groups in neutral physiological environments. A biodegradable scaffold formed via radical polymerization crosslinking of methyacrylamide-modified chitosan allowed neurites to penetrate the construct, and covalent modification with maleimide-terminated cell adhesive peptides mi-GDPGYIGSR and mi-GQASSIKVAV to thiolated forms of the scaffold enhanced cell adhesion and the average neurite length.[61] In another study, the cell survival properties in thermally gelling chitosan hydrogels were optimized through poly-D-lysine immobilization, which produced neurons exhibiting larger cell bodies, single neurite extensions, and enhanced cell survival.[37]

Biologically derived hydrogel polymers are commonly used for cell culture studies. Matrigel is one such commercial hydrogel made from ECM extracted from Engelbreth–Holm–Swarm (EHS) sarcoma containing laminin, fibronectin, and proteoglycans.[62] An in vitro study where Matrigel was seeded with a co-culture of neurons and astrocytes yielded extensive 3D neurite outgrowth and expression of mature neuron-specific cytoskeletal proteins, and produced a network of functional synapses, as confirmed by patch clamping.[63] Matrigel added to collagen scaffolds also supported Schwann cell proliferation and neurite formation.[64] In contrast, when Matrigel was tested using human neural progenitor cells, the cells' normal capacity for differentiation was hindered.[65] However, the animal origins of Matrigel constituents render these scaffolds unsuitable for deployment in humans owing to the potential of disease transmission and immunorejection issues. Although present research utilizes animal cells and models, the transferability of these experiments to humans remains to be explored.

### *Synthetic Hydrogels*

Generally, synthetic hydrogels are biologically inert and therefore have weak cell adherence. However, synthetic hydrogels are commonly chemically stable and can be optimized for neural engineering applications. Modified synthetic hydrogels circumvent some drawbacks associated with natural polymers as superior tuning of mechanical properties can be obtained, while the lack of biofunctionality can be addressed through the tethering of cell adhesive peptide motifs and/or the incorporation of natural polymers.

Several synthetic hydrogels such as poly(*N*-2-(hydroxypropyl)methacrylamide) (pHPMA),[34,66] poly(hydroxyethylmethacrylate) (pHEMA),[67] and polyethylene glycol (PEG) have been used for the repair of brain lesions. Hydrogels formed from pHPMA and pHEMA cannot gel in situ and therefore must be implanted preformed, thereby necessitating invasive surgery.[3] Nonetheless, some of these hydrogels have produced promising results. A macroporous pHPMA prepared by heterophase separation using radical polymerization in a pore-forming solvent with a divinyl crosslinking agent was capable of bridging a brain lesion while supporting cell penetration, angiogenesis, axon growth, and ECM formation within the scaffold.[34,66,67] In contrast, after implantation of pHEMA into the injured brain, only astrocytes penetrated the hydrogel,[67] illustrating the diversity of these synthetic polymers. Furthermore, electrically conductive hydrogel blends such as poly(HEMA)-based hydrogels with polyaniline and poly(HEMA)-based hydrogels with polypyrrole, have also been fabricated[68] that can potentially be applied in neural tissue engineering applications where electrical stimulation of neural cells can be exploited.

An example of a polymer conjugate used in neural engineering is PEG and polylysine. PEG is a low-toxicity polymer reported to repair and protect cells following spinal cord lesions.[62] A photopolymerized hydrogel composed of a polylysine macromer backbone with linear PEG branches supported the survival and proliferation of neural progenitor cells in vitro and also biased their differentiation towards mature neurons.[69] PEG–polylysine hydrogels of various elastic moduli elicited different stem cell responses, with low modulus gels between 3.5 and 5.5 kPa facilitating cell migration, and endorsing neural differentiation.[70] Star-shaped PEG was also used to form a biohybrid hydrogel through covalent crosslinking with heparin and biofunctionalization by tethering RGD peptide and fibroblast growth factor-2 (FGF-2) via secondary conversion of heparin.[71] Variation in the properties of this scaffold such as mesh size, swelling, and elastic modulus also influenced cell traits in a co-culture of primary nerve cells and stems cells.

Various protein and polysaccharide–polymer bioconjugates have also been investigated in relation to brain repair. Tailoring the modulus of polyacrylamide hydrogels through crosslink density and functionalizing with covalently bound fibronectin impacted neurite formation such that a soft gel (~10 Pa) yielded

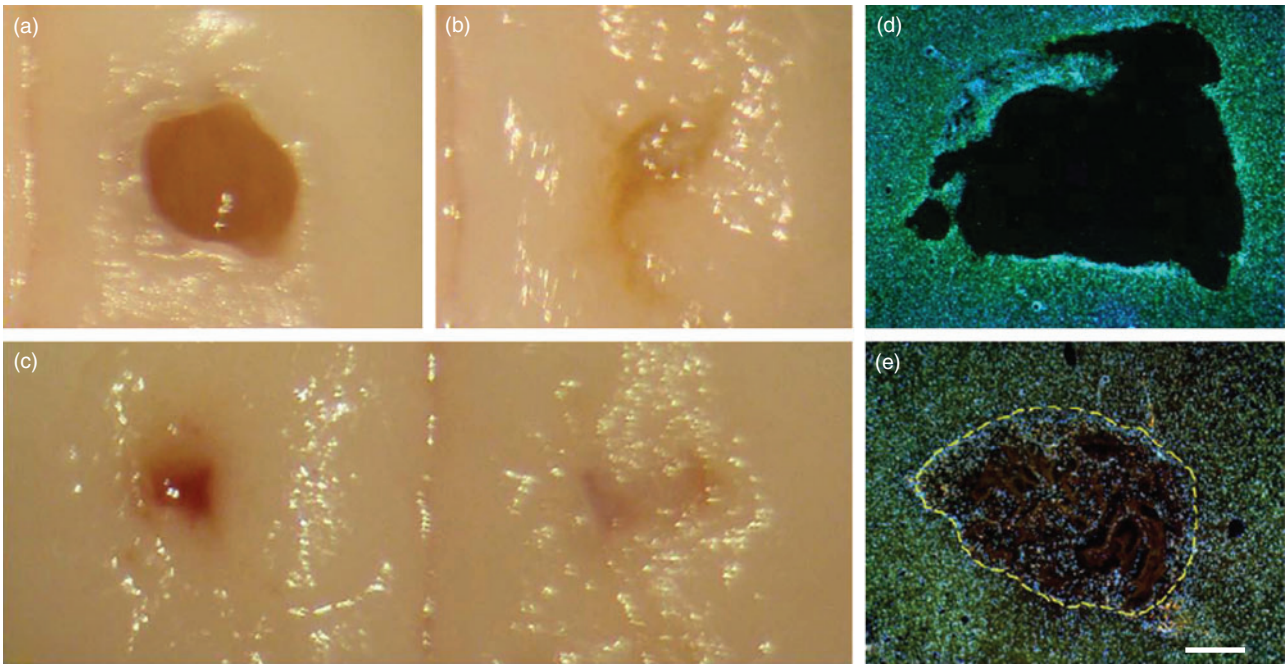


**Fig. 3.** Brain injury lesions with different treatments 6 weeks after surgery. (a) Lesion cavity created by saline injection; and (b) closed wound after self-assembling peptide nanofibre scaffolds (SAPNS) treatment; (c) bilateral brain injury illustrating saline treatment in the left hemisphere and SAPNS treatment in the right; (d) Nissl and DAPI double staining depicting saline-treated lesion; and (e) SAPNS-treated lesion that has integrated well with host tissue. Scale bar: a–c = 1 mm; d, e = 500 μm. Reprinted from ref. [78], with permission from Elsevier.

few, unbranched, short neurites whereas stiffer substrates (1 to 100 kPa) yielded longer and branched neurites.[35] Such two-dimensional mechanotransduction studies provide considerable insight into tailoring scaffolds for brain tissue engineering; however, 3D models may yield different results and provide a more useful tool for translation to animal models.[72]

Hydrogels demonstrate a capacity to encapsulate cells, regulate their behaviour, and facilitate integration into host tissue. Further optimization of hydrogels for enhanced cell interactions and cell penetration through biomolecule functionalization is necessary before they can be deployed in vivo to promote neural repair. In addition to their neuron regeneration capacity, the future of hydrogels will also need to assess the functional recovery this form of therapy offers as the ultimate aim would be to reverse not only the structural damage to the brain, but also restore the lost cognitive, sensory, and motor functions.

## Self-assembling Peptides

An alternative form of hydrogel for brain tissue engineering are self-assembling peptide nanofibre scaffolds (SAPNS). These hydrogels are manufactured from various oligopeptides or amphiphilic molecules that spontaneously aggregate to form nanofibres, which subsequently form a fibrillar network in the presence of physiological ionic conditions.[73] Amphiphile peptide molecules form nanofibres that are composed of a core of hydrophobic tails while the hydrophilic head-groups form a sheath.[73] SAPNS are characterized by high porosity, tissue-like water content, and enhanced cell signalling by high-density presentation of bioactive peptide sequences.[74] However, the high water content renders SAPNS mechanically weak and the biological origins increase susceptibility to enzymatic degradation in vivo.

SAPNS used in neural tissue engineering have predominantly involved two types of polymer peptides – an ECM-derived sequence isoleucine–lysine–valine–alanine–valine (IKVAV) and arginine–alanine–aspartate–alanine (RADA)16-I. IKVAV SAPNS induced selective differentiation of encapsulated neural progenitor cells into neurons while downregulating astrocyte differentiation.[74,75] This feature has been attributed to the capacity of SAPNS to amplify presentation of the neurite-promoting laminin epitope, IKVAV, on the surface at van der Waals packing distances.[75] Further work on IKVAV-functionalized scaffolds has resulted in their implantation into spinal cord injury models, with some degree of tissue regeneration and functional recovery being exhibited in mice.[76] In contrast, RADA16-I SAPNS supported cell attachment, differentiation, and neurite outgrowth in vitro and functional synapse formation in situ without eliciting an immunogenic response.[77] Application of SAPNS in brain lesions virtually eliminated cavitation, with fewer astrocytes and macrophages present at the lesion site indicating low immunogenicity compared with controls exhibiting secondary tissue loss.[78] Fig. 3 depicts post-surgery healing of brain lesions treated with SAPNS. RADA16-I SAPNS is also permissive to axonal growth such that neural tracts could be partially restored and functional recovery attained after brain injury.[79,80] Although these primary in vivo studies provide promising results, a deeper understanding and optimization of SAPNS and its interactions with neural tissue is necessary. SAPNS for use in neural tissue engineering, let alone the brain, is still in its infancy.

## Electrospun Nanofibres

Electrospun scaffolds consist of a nanofibrous mesh formed by uniaxial stretching of a viscoelastic polymer solution under an applied voltage. The application of a voltage instigates charge

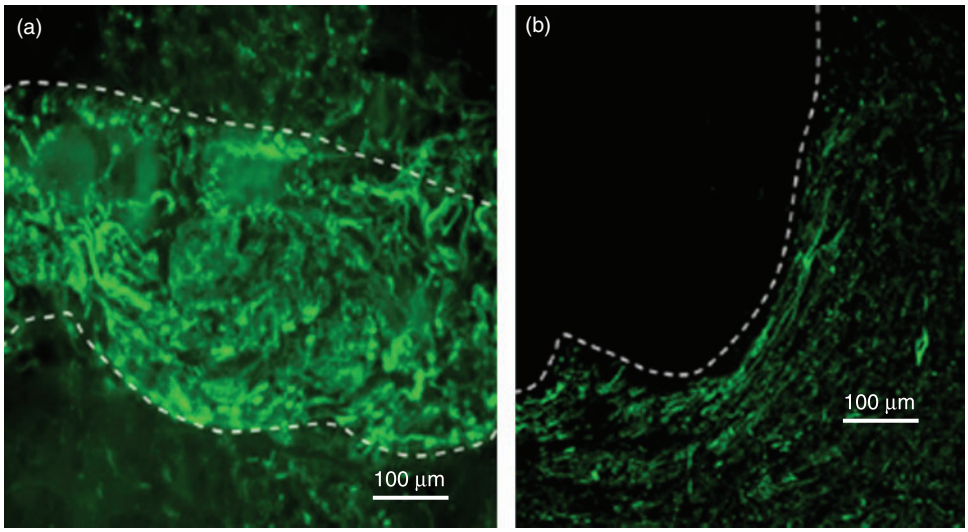


**Fig. 4.** (a) Fluorescent-stained images of neurite infiltration on a randomly orientated fibre scaffold; and (b) a partially aligned fibre scaffold 60 days after implantation. Scaffolds not imaged. Reprinted from ref. [1], with permission from Elsevier.

accumulation to counteract the solution's surface tension, resulting in the formation of a Taylor cone.[81] At a critical voltage, a polymer jet is ejected from the cone tip and accelerated towards a collector. As the jet travels, whipping instabilities draw out the fibre to nanoscale diametres.[82] There are several electrospinner configurations available today; however, the mode of fabrication is irrelevant in the context of brain repair and readers are referred to the following articles for more information on conventional electrospinning.[81,83]

Interest in nanofibrous scaffolds for tissue engineering is based on the structural similarity of the electrospun nanofibres to the hierarchical fibrillar arrangement of collagen, laminin, and other fibrils of the ECM.[84–86] The fibre diameters of electrospun scaffolds typically range from a few nanometres to 1 μm.[85] From another perspective, nanofibres mimic other ECM attributes such as a large surface area-to-volume ratio, high porosity, and similar mechanical properties.[86] High porosity and fibrillar traits facilitate cell and axon penetration, neurite contact guidance and diffusion of nutrients and waste, all of which act to enhance scaffold–tissue integration. It is also noteworthy that aligned fibrous scaffolds prepared by electrospinning have demonstrated a capacity to orient neurite growth through parallel[87–89] and perpendicular[1] contact guidance.

A variety of polymers have been electrospun for neural tissue engineering applications, including: poly(ε-caprolactone) (PCL),[1,90–93] poly(lactic-co-glycolic acid) (PLGA),[94] polypyrrole,[94] polylactide (PLA),[93] polymethyl methacrylate (PMMA),[50] and polyacrylic acid (PAA),[50] to name a few. PCL, PLGA, and PLA are commonly used as these polymers are biodegradable via hydrolysis of the ester linkages and have approval from the Australian Therapeutic Goods Administration and the US Food and Drug Administration for use in biomedical applications. The presence of ester linkages in the polymer backbone also provides a convenient means by which they can be biofunctionalized by covalent conjugation with various biomolecules. This also applies to PAA, where the acrylic acid can be esterified or aminated to allow bioconjugation. Polypyrrole is a highly conductive polyacetylene derivative that is becoming increasingly employed owing to its potential to stimulate signal transduction in neural cells.[94]

Randomly orientated and aligned PCL scaffolds were used to develop a system that simulates brain tumour migration in vitro.[90] Glioma (tumour) cells exhibited faster migration on aligned scaffolds, as the tortuous paths in random scaffolds are likely to decelerate cell migration. However, when random and partially aligned electrospun PCL scaffolds were implanted in the adult rat brain to study endogenous cell migration, neurites existing at the scaffold–tissue interface displayed perpendicular axon guidance on partially aligned electrospun scaffolds, whereas a random scaffold promoted neurite penetration,[1] as depicted in Fig. 4. Contrasts in findings between such studies represent the dualities encountered in designing scaffolds that facilitate rapid cell migration and penetration. Porosity plays an important role in enabling cell penetration of the scaffolds; however, the tortuous paths it creates delay axon traversal of the scaffold. Furthermore, this also exemplifies the difference in cellular responses to in vitro and in vivo environments, thus emphasizing the insufficiency of in vitro models alone in assessing the functionality of scaffolds.

Electroactive scaffolds that can potentially facilitate communication between neurons in the brain have been of recent research interest. PCL and poly-L-lactide nanofibrous scaffolds were coated in polypyrrole via in situ polymerization to form conductive sheaths.[93] Dorsal root ganglion cells produced neurites of greater length on both random and aligned scaffolds when subjected to electrical stimulation (random $= 1730 \pm 140\,\mu m$, aligned $= 2540 \pm 170\,\mu m$) compared with no stimulation (random $= 950 \pm 160\,\mu m$, aligned $= 1720 \pm 340\,\mu m$). A similar scaffold composed of polypyrrole-coated PLGA also enhanced neurite formation and neurite lengths on electrical stimulation.[94] The properties of nanofibrous scaffolds can also be enhanced through the attachment of biomolecules such as collagen to the surface to improve cell viability and attachment.[50]

## Functionalizing Scaffolds for Brain Tissue Engineering

Biomolecules form an integral part of neural regeneration through the regulation of cell adhesion, proliferation, migration, and differentiation. In vivo, the type of biomolecules,

their form (soluble or insoluble), conformation and quantity all influence the responsiveness of cells.[95] Consequently, it is necessary to have a biologically relevant molecular support system incorporated into a scaffold to facilitate neural regeneration.

Incorporation of biomolecules into a scaffold is generally a post-manufacture treatment strategy because solvents used to dissolve polymers, during electrospinning, are detrimental to biological substances. Core-shell[96,97] and emulsion electrospinning[98] provide a means of circumventing these problems by enabling the formation of tubular fibres capable of encapsulating biomolecules or eliminating the need for solvent use.[99] However, biomolecules are commonly covalently attached to scaffold surfaces to maintain the mechanical integrity of the polymer while imparting biological properties. It also prolongs the lifespan of the molecule in a given region by avoiding phagocytosis, thereby sustaining activation of signalling pathways.[100] However, retention of bioactivity by tethering may be lost as the functionality of many growth factors and proteins is conformation- and orientation-dependent. It is difficult to orchestrate the tethering process to retain bioactivity owing to the presence of multiple target functional groups in biomolecules that can react during the process. Therefore, incorrect orientation of the biomolecule can render the scaffold non-biofunctional or functional to a lesser extent than the soluble form.[101]

The incorporation of essential ECM proteins into scaffolds has been demonstrated as a means of enhancing biocompatibility. Various other proteins and ligands have also been grafted or adsorbed onto scaffolds, in particular neurotrophins and factors associated with neural regeneration. Table 1 outlines some biomolecules that have been coupled to scaffolds with potential for neural engineering applications. These biomolecules are also being used to create concentration gradients within scaffolds to ensure appropriate spatial migration and differentiation of neural stem cells and their axonal growth.[102,103]

## Clinical Applications

Treatment strategies for many neurodegenerative disorders or neurotraumas are limited and commonly rely on pharmacological intervention, physical therapies, and some surgical intervention. Unfortunately, many of these treatments have little effect on disease and injury modification. In this regard, cell transplantation therapies, to replace lost neurons, offers more long-term hope. It is probable and likely that a 3D scaffold milieu for the attachment and organization of cells, as well as the support of neuritic processes, will improve cell integration in the host. Furthermore, neurotrophic and anti-apoptic factors may promote the survival, proliferation, and differentiation of neural progenitor cells, facilitating transplant or endogenous repair processes.[112] In order to address this requirement, scaffolds are currently being designed, fabricated, and assessed as tissue regenerative implants, incorporating these biologically relevant modifications.

Scaffolds intended for the replacement, repair, and regeneration of damaged brain tissue must be designed to possess key features of brain tissue in order to accommodate graft–host integration. Furthermore, an implantable scaffold also must be customised to address issues specific to the neural condition as the pathophysiology will differ for each condition and affect neural cytoarchitecture in a different manner. Here, a brief discussion of how tissue engineering can contribute to therapeutic treatment, specifically for TBI, will be discussed as a case study.

### Traumatic Brain Injury

TBI can occur in many ways; however, typically the brain ricocheting inside the skull during impact inflicts the most damage. Symptoms of TBI are highly diverse, ranging from headaches and dementia through to severe impediments such as paralysis. Irrespective of the primary injury, damage to the brain initiates complex cellular and biomolecular mechanisms that evolve over a lengthy time period, resulting in neuronal cell death.[113] The type of primary injury underpins the ensuing cascade of pathophysiological events, i.e. whether it is a lesion or application of a force. However, the main obstacles to regeneration include the formation of voids in tissue due to neuron degeneration,[114,115] scar tissue formation,[114,116] release of inhibitory axon growth factors,[113–115,117] and failure of neurons to initiate axon regeneration.[114,115,117]

Treatment strategies for TBI aim to minimize further injury, as the initial brain damage cannot be reversed.[113] Immediate treatments focus on ensuring oxygen supply to the brain, maintaining adequate blood flow and controlling blood pressure to maintain organ viability. Severely injured patients require surgery to remove haematomas (ruptured blood vessels) or repair contusions (damaged tissue).

Implantable scaffolds could aid patients requiring surgery after a TBI where damaged tissue can be replaced with a biomaterial construct. This will facilitate surrounding tissues maintaining their architecture and promote tissue regeneration. The scaffold could potentially carry neural stem or progenitor cells that differentiate into the appropriate neural lineage with stimulation and/or facilitate endogenous cell infiltration and reformation of the neural network. Thus, it may be feasible to utilize a scaffold in TBI treatment to structurally support the endogenous tissue and prevent tissue collapse, as well as to provide cells with an artificial ECM network.

## Challenges in Brain Tissue Engineering

Tissue-engineered scaffolds offer some prospects for the treatment of neurodegenerative disorders and brain injuries. At present, the design and fabrication of these scaffolds is still in its infancy and must overcome several hurdles before their employment in treatment schemes. As scaffolds are designed to support neural cells (either transplanted or endogenous), the major obstacles relate to moderating cell function through optimization of surface functionality, mechanical properties, and biological activity. Therefore, the requirements of a scaffold to form a cellular microenvironment also translate to challenges for tissue engineers. Challenges encountered by scaffolds include limitations in porosity, pore dimensions, three-dimensionality, and surface functionality. Issues relating to toxicity of residual solvents, mechanical compatibility, biocompatibility, and degradation traits also need to be taken into consideration.

Fabricating a scaffold that overcomes these morphological and biological limitations is a challenging task. It may not be feasible to manufacture a scaffold that incorporates all these features; however, it is necessary to impart an optimal degree of interconnected porosity, neurotrophic cues, and mechanical compatibility in order to maximize benefits for in vivo applications. Moreover, the biological activity of the scaffold should be tailored to address the necessities of individual neurological conditions due to differences in the pathophysiology.

Beyond the current iterative process of optimizing scaffolds for in vitro cell growth, the future challenges in engineering

**Table 1. Biomolecules tethered onto scaffolds for potential brain-tissue engineering applications**

Note that some studies above do not pertain to brain tissue regeneration *per se*; however, the findings may be transferable brain tissue engineering

| Biomolecule | Scaffold | Attachment method | Cell responses | Reference |
|---|---|---|---|---|
| RGD | Hyaluronic acid hydrogel | Covalent coupling via 1,1′-carbonyldiimidazole activation | – Support cell infiltration<br>– Promote angiogenesis<br>– Inhibit glial scarring<br>– Promote neurite extension | [49] |
| Arginine–glycine–aspartate–serine peptide (GRGDS) | Agarose hydrogel | Photoirradiation of hydrogel modified with *S*-2-nitrobenzyl-cysteine | – Cells only grew within modified channels<br>– Cell migration and process extension limited to modified regions | [104] |
| Vascular endothelial growth factor (VEGF) | Polydimethylsiloxane–tetraethoxysilane scaffold prepared via sol-gel using sucrose particles as porogen | Hydrophilic affinity | – New tissue formed comprising astrocytes and endothelial cells<br>– Vascularisation evident | [105] |
| Collagen | Methyl methacrylate and acrylic acid nanofibres | Soaking in peptide solution in the presence of 1:1 EDC/NHS | – Enhanced attachment and viability | [50] |
| Peptides mi-GDPGYIGSR and mi-GQASSIKVAV | Methacrylic chitosan hydrogel | Covalent binding through thiolation of scaffold | – Degradation of scaffold via lysozyme action<br>– Neurite infiltration<br>– Improved cell attachment | [61] |
| FGF-2 | Polyacrylamide-based hydrogel | Adsorption | – Maintained undifferentiated state | [106] |
| | Polyamide nanofibres | Covalently coated with a proprietary polyamide polymer and crosslinked via sulfo-LC-SDPD | – Enhanced FGF-2 expression in cells<br>– Enhanced stellate morphology of astrocytes<br>– Scaffold more permissive to neurite growth | [107] |
| Ciliary neurotrophic factor (CNT) | Polyacrylamide-based hydrogel | Adsorption | – Expression of glial fibrillary acidic protein (GFAP)<br>– Increasing concentration increases GFAP expression | [106] |
| Fetal bovine serum (FBS) | Polyacrylamide-based hydrogel | Adsorption | – Expression of smooth muscle actin (SMA) | [106] |
| Neurite growth factor (NGF) | Polypyrrole film | Immobilized via intermediate polyallylamine crosslinker exposed to UV light to activate azido groups | – Did not affect cell growth | [108] |
| | Poly(2-hydroxyethyl-methacrylate) (p(HEMA)) | Dissolution in hydrogel solution | – Cell neurite guidance up gradients | [109] |
| Laminin | Collagen-I hydrogel on glass coverslip | Microcontact printing | – Neurites extended at the gel surface | [110] |
| Chondroitin sulfate proteoglycans | Collagen-I hydrogel on glass coverslip | Microcontact printing | – Neurites extended into the gel | [110] |
| Leukaemia inhibitory factor (LIF) | Poly(octadecene-*alt*-maleic anhydride) copolymer film | Covalent attachment through PEG7 spacer arms, and non-covalent bonding | – Supported stem cell pluripotency for 2 weeks | [111] |
| BDNF | Poly(ε-caprolactone) | Covalent immobilization through aminolyzation and tethering with sulfo-(succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate) | – Enhances stem cell proliferation<br>– Directs differentiation towards neural and oligodendrocyte linages | [92] |

scaffolds for brain injuries involve promoting integration of the construct with the native tissue and achieving functional recovery. Although there are some in vivo investigations ascertaining scaffold biocompatibility and identifying the cell types that infiltrate the construct, the functional nature of the regenerating tissue remains to be explored. In vitro patch-clamping studies have determined functional neuron cell signalling capacities on scaffolds; however, a signal transmission capacity alone may be insufficient in the brain where precise reconnection into the overall neural circuitry is also required for complete restoration of function. Therefore, guiding the growth of neural processes also needs to be addressed.

## Conclusion

The complexity of the brain and the myriad of biomolecular and signalling cascades associated with the pathology of neurological disorders present intricate challenges to formulating treatment strategies. Cell-based therapies have been found to initiate restoration of neurological cells in the damaged site to a limited extent and found to facilitate cognitive function. A variety of scaffolds have been engineered to provide an artificial microenvironment for enhanced cell survival, proliferation, and migration in 3D. Hydrogels, SAPNS, and nanofibrous scaffolds have been investigated for potential in vivo application in the repair, replacement, and regeneration of damaged brain tissue. Each scaffold variant possesses favourable attributes and limitations that must be attuned to optimize the morphology and bioactivity of the construct to promote cell penetration and host tissue integration. Imparting bioactivity into scaffolds is essential to enabling cell–matrix and cell–cell interactions. This has been achieved through the attachment of biomolecules such as ECM proteins and trophic factors to direct cell development and proliferation. It is increasingly evident that scaffolds must embody key features to promote transplanted cell survival and host cell integration as well as address some aspects of pathophysiology of the condition through an individualistic disorder-based approach. A biofunctionalized hydrogel, self-assembling scaffold, or electrospun scaffold is likely to be insufficient in addressing the design criteria. A sophisticated hybrid scaffold may instead be the key for promoting neural repair. In terms of scaffold development, research is still in its early stages and more iterative work comprising both in vitro and in vivo animal studies is necessary before clinical trials. However, the employment of scaffolds in repairing damaged brain tissue is likely to be a feasible treatment option.

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