Scaffolds for brain tissue reconstruction

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34.1 Overview of brain reconstruction: scaffold types and design objectives

The brain is the most complex organ in the human body, functionally comprised of over 100 billion nerve cells, called neurons, that are precisely interconnected by a sophisticated network of over 100,000 miles of fibrous extensions, called axons [1,2]. These exquisite neuronal–axonal networks are complimented by an array of specialized support cells, collectively called glia, that are 10 times more numerous than neurons yet fulfill necessary roles including providing structural, trophic, metabolic, and functional support for neurons and axonal tracts [1]. The focus of this chapter is on brain repair, specifically emerging strategies to facilitate regeneration, reconnectivity, and/or reconstruction of these exquisite neuronal–axonal–glial networks following degeneration caused by brain injury or neurodegenerative disease. Focus is given to engineered scaffold-based approaches; in particular, efforts encompassing naturally occurring organic/biologically derived scaffolds (e.g., extracellular matrices (ECMs)), non-ECM polymer-based scaffolds (e.g., hydrogels), and so-called "living scaffolds" (e.g., anisotropic cell-laden scaffolds). In general, these biomaterial/biological scaffolds aim to fill defects, provide structural support, provide guidance cues (generally anisotropic), deliver biological agents, and/or provide preorganized cells. The design criteria for these scaffolds are generally determined by the desired mechanism(s) of action, which for brain repair generally fall into three categories: (1) *neuroprotection* by providing prosurvival cues (growth factors) and/or supporting cells; (2) *cell replacement* by eliciting neurogenesis, glial proliferation, orchestrating (endogenous) cell migration, and/or providing new (exogenous) cells, with varying degrees of three-dimensional (3D) architecture; and (3) *neurite pathfinding* (generally axonal) to restore local connections and/or reconstruct long-distance circuitry. The ultimate goal of these therapies is to prevent cell/axonal loss, promote healing, reconstruct lost neural architecture/connections, and ultimately facilitate functional recovery following nervous system disorders.

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34.2 Nervous system function, anatomy, and cellular architecture

Effective strategies to facilitate brain reconstruction require an appreciation of the sophisticated architecture (spanning micro- to macroscales), multicellular composition, and unique functional domains found across the nervous system [1,3]. In total, the nervous system serves as the body's communication and control network. A complex collection of specialized cells gathers and processes information from various sources, routes the information to the appropriate locations, and generates responses to the information to elicit a range of physiological functions [1,4,5]. The information may relate to the external environment, the body's position and orientation in space, or various tissue, organ, and system-level phenomena [1,5]. Functionally, the nervous system is subdivided into the somatic (associated with conscious control such as movement) and the autonomic (responsible for unconscious/involuntary behavior such as breathing or digestion) nervous systems [1,6]. Anatomically, the nervous system has two primary components—the central nervous system (CNS), comprises the brain and spinal cord, and the peripheral nervous system, encompassing the nerves that bridge the CNS to the rest of the body [1].

The focus of this chapter is on the brain, which serves as the primary mammalian processing and coordination center. Information about the environment (i.e., external stimuli) and the body's structural and functional behavior (e.g., proprioception) is encoded as electrical signals that are transmitted along axons—long fibers or tracts projecting from neurons—for feature extraction, processing, and integration and redirection to appropriate subnetworks within the brain [1]. Similarly, output signals from the brain are delivered along axons to either influence physiological phenomena or execute voluntary actions [7]. Structurally, the brain is segregated into gray matter (populated by neuronal cell bodies and supporting glia) and white matter (the collective bundles of axonal pathways that connect the brain to itself and the rest of the nervous system) [8]. This functional architecture enables rapid communication between areas that may be separated by several centimeters, as well as multiple feedback loops and subnetworks that both affect and are affected by neural activity [9,10]. There is also the blood–brain barrier (BBB), which derives from special properties of endothelial cells lining the vasculature in the brain; the BBB effectively restricts which components from blood are permitted to enter into the intracellular and/or interstitial space in the brain [11,12]. Multiple layers of connective tissue also protect the entire brain [1,11,12].

There are two main types of cells in the brain—neurons, the primary information carriers and functional cells, and glia, which maintain the microenvironment necessary for neuronal survival and provide support for various neuronal functions [1]. Both types have a substantial range of subtypes categorized by their localization, specialized functions, gene expression, and phenotype. Broadly, neurons possess multiple structures for receiving and processing inputs from other neurons (dendrites) and an output structure (axon) for transmitting signals [1]. Although neurons generally possess a single axon, axons may branch into multiple output terminals to deliver

information signals to multiple targets through synapses, which are nanoscale junctions where signal transfer occurs between a neuron and an end target [3,13]. At these synapses, neurons release neurotransmitters to increase, inhibit, or otherwise modulate the activity of downstream cells; glutamate, for example, primarily excites neurons, whereas GABA reduces their potential for activation [14]. Glial cells in the brain consist predominantly (though not exclusively) of astrocytes, oligodendrocytes, and microglia [3]. Astrocytes regulate physical and chemical homeostasis, for instance, through production and maintenance of various elements of the ECM and BBB [15]. Astrocytes also regulate several signaling pathways critical for neuronal function, energetics, and synaptic efficacy [15]. Oligodendrocytes are the primary myelinating cells in the CNS; myelin is the fatty insulation surrounding axons that permits rapid conduction of electrical impulses, termed action potentials [1,16]. Microglia act as the primary immune cells of the brain, targeting foreign bodies and degrading cellular debris following neural injury and during subsequent remodeling [15].

Several features of the neuroanatomy and architecture of the brain present a host of nontrivial design challenges for reconstruction strategies (Fig. 34.1). These features include the brain's multicellular composition (e.g., neurons, astrocytes, oligodendrocytes, microglia), the existence of multiple neuronal (e.g., GABAergic/inhibitory, glutamatergic/excitatory, dopaminergic/modulatory, etc.) and glial subtypes, the organization of these cells into distinct networks, structures, and domains (e.g., the six layers of the neocortex), and the long-distance axonal pathways spanning these structures that often project several centimeters and may overlap with other pathways throughout the brain [1,3,6,7,9,14,17,18].

34.3 Injury, neurodegeneration, and endogenous barriers to regeneration

Any attempt to facilitate repair and regeneration of the brain requires a deep understanding of the neural responses to neurotrauma and/or neurodegenerative disease as well as endogenous barriers to regeneration. The brain is susceptible to damage through mechanical disruption of neural cells and axonal pathways, as seen in traumatic brain injury (TBI), hypoxic-ischemic insults as found in stroke, and the chronic neurodegenerative sequelae of diseases such as Alzheimer's disease or Parkinson's disease (PD), among numerous other neurological afflictions [19–24]. Whether due to neuronal/axonal loss or dysfunction, this damage may result in negative clinical outcomes including cognitive deficits, memory impairment, lost or aberrant movement, and/or personality changes [25,26]. Depending on the localization and severity of the insult, multiple areas of the brain may be affected, compounding the complexity and extent of the damage. Furthermore, compared with other organ systems, the brain is limited in its ability to recover following cellular degeneration or axonal loss due to the physical complexity of reconstructing sophisticated neural architectures and distant neural connections, a limited ability to generate new neurons, and an environment that in general is inhibitory to axonal outgrowth (effectively antiregenerative) [27–29]. Indeed, whereas the

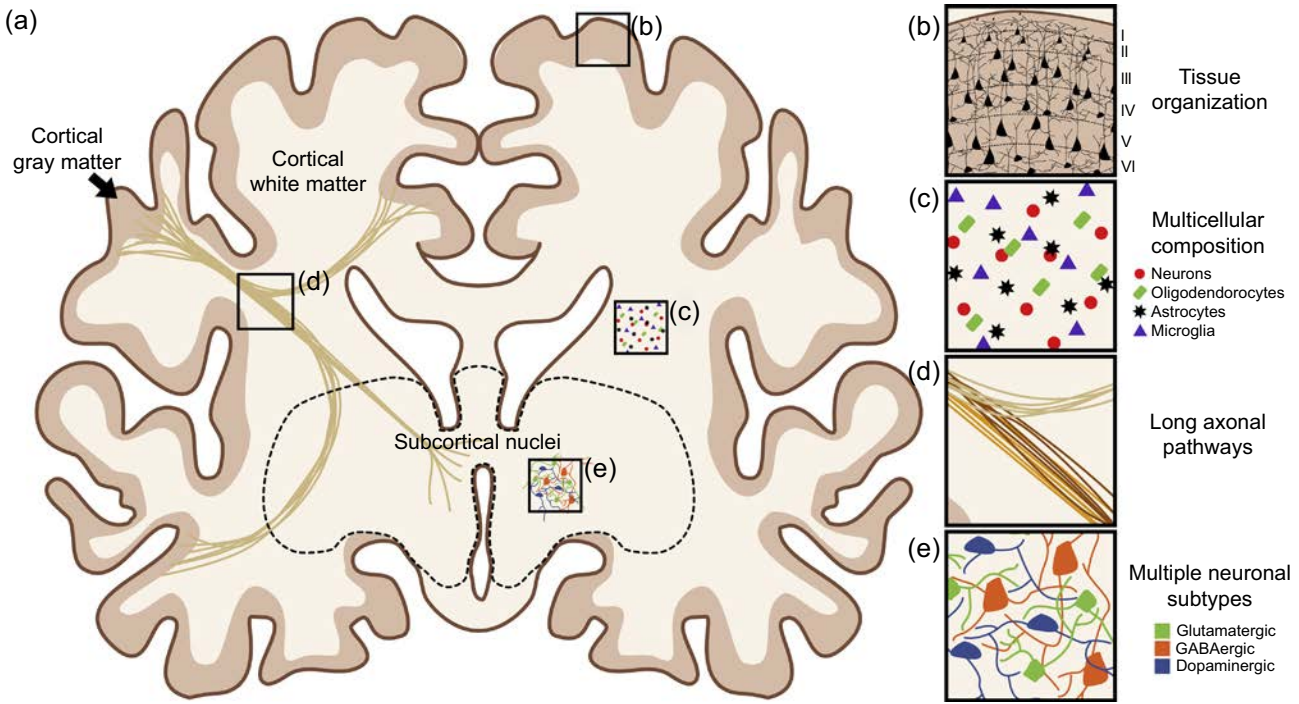

Figure 34.1 Requirements for brain tissue scaffolds. Anatomical features and challenges in brain tissue reconstruction include (a) the segregation of gray and white matter, (b) the organization of neuronal/glial subtypes within discrete structures, (c) the presence of multiple cell types, (d) the long bundles of specialized axonal tracts, and (e) the importance of circuits composed of multiple neuronal subtypes.

physical and chemical microenvironment of the brain enables neuronal birth, cell migration, axonal outgrowth, and large-scale synaptic connectivity during embryogenesis, the milieu of the adult brain transitions from promoting growth to maintaining the overall form and function of its complex mature architecture. As such, the microenvironment of the adult brain inhibits the formation and growth of new neurons outside of a few specialized niches. The predominant exceptions are the subventricular zone (SVZ) and the subgranular zone (SGZ). Neural stem cells in the SVZ give rise to progenitors that migrate along the rostral migratory stream to form new interneurons in the olfactory bulb, whereas those in the SGZ give rise to dentate granule neurons in the hippocampus [30–32]. The loss of similar pathways after embryogenesis prevents the migration of new neurons to other areas that may have undergone neuronal loss/attrition in adulthood [32–34]. Although there is evidence that neural progenitors from the SVZ migrate to lesions acutely following injury, these progenitors were shown to differentiate into other cell types or remain undifferentiated rather than produce new neurons [31]. In general, aside from a few limited niches in the brain, there is minimal endogenous capacity to regenerate neurons lost due to brain injury or neurodegenerative disease.

Similarly, although guidance cues in the developing brain direct axonal growth and connectivity in a relatively small volume, those cues are largely lost in the mature brain, which ultimately comprises an extensive volume with mature axon pathways spanning several centimeters [1]. Moreover, axons generally become myelinated postnatally; as such, axon degeneration in adulthood generally results in the release and subsequent diffusion of myelin fragments that are thought to be potently inhibitory, thereby providing a further barrier to long-distance axon regeneration [35–37]. Overall, after axonal loss, there is minimal functional axonal regeneration due to vast distances to appropriate targets, loss of guidance cues, and the presence of inhibitory signals. The loss of seemingly irreplaceable neurons also obviously reduces the capacity for axonal regrowth. Collectively, these factors limit the potential for damaged axons to regrow to reach their proper targets. Indeed, long-distance axonal connections in the brain lost due to trauma or disease are generally considered nonrecoverable absent the application of exogenous engineered solutions.

In addition to degenerative changes, following injury or disease, the brain's immune response may result in a proinflammatory environment. Astrocytes and microglia may be recruited to the affected site(s), where they shift from a resting to active phenotype; this state is characterized by the release of cytokines, chemokines, neurotransmitters, and reactive oxygen species [38,39]. There may also be an infiltration of circulating peripheral immune cells, generally unable to cross the BBB, such as monocyte-derived macrophages [40,41]. These endogenous processes eliminate debris from degenerating cells/axons (and foreign bodies if necessary) and limit the spread of further immediate damage [29,40]. However, these reactive responses generally occur at the cost of limiting the potential for regeneration [19]. Indeed, longer-term processes following neural damage may limit recovery; for instance, glial scarring, a common reaction to focal injury in the brain, is carried out and sustained predominantly by astrocytes, which form a physical and chemical barrier around the injury site [39,42]. Although glial scars sequester the focally damaged area, they also prevent local axonal growth due to the fibrous matrix surrounding the injury [43].

34.4 Overview of current scaffold-based approaches for neuroregeneration

This chapter provides an overview of engineered scaffold-based approaches to facilitate brain repair. In particular, we describe efforts encompassing naturally occurring organic/biologically derived scaffolds (e.g., ECM-based scaffolds), nonorganic polymer-based scaffolds (e.g., hydrogels), and so-called "living scaffolds" (e.g., anisotropic cell-laden scaffolds). The design criteria for these scaffolds are generally determined by the desired mechanism(s) of action, which for brain repair generally falls into three interrelated categories: (1) *neuroprotection* by providing prosurvival cues (growth factors) and/or supporting cells; (2) *cell replacement* by eliciting neurogenesis, glial proliferation, orchestrating (endogenous) cell migration, or providing new (exogenous) cells, with varying degrees of 3D architecture; and (3) *neurite pathfinding* (generally axonal) to restore local connections and/or reconstruct long-distance circuitry.

34.4.1 Design considerations

The scaffold type, constituents, and timing of delivery must be carefully considered based on the design objective(s) and desired mechanism(s) for brain repair. For example, if the goal is to attenuate degeneration following brain injury, then a treatment should be added in the acute or subacute phase before the majority of cell/axonal loss. However, if such an acute therapy involves cell-laden scaffolds (e.g., delivery of neural stem cells to provide multifaceted neurotrophic support), then the behavior of these cells in the "hostile" environment of active degeneration and inflammation must be considered. Similarly, if a bioactive scaffold is used as a means for drug delivery (e.g., for controlled release of prosurvival or antiinflammatory factors), then the degradation profiles in a rampantly inflammatory environment should be considered. For instance, for scaffolds and other implants, the glial scar may prolong the release of proinflammatory factors; this effect has been shown to depend on the degradability profile of the implant [39]. Thus, acute pathophysiological responses can reduce the efficacy of acellular and/or cell-seeded scaffolds by drastically affecting scaffold degradation time and exposing cells to detrimental cytokines associated with an inflammatory environment, thereby affecting scaffold efficacy and implant cell survival. However, provided that these factors are adequately accounted for in scaffold design and optimization, this acute implant period following injury can be extremely impactful—and set the stage for further regeneration—by limiting the scope of degeneration and hence tissue loss.

Complimentary to the goal of neuroprotection, longer-term strategies generally involve the objectives of cell replacement and/or axonal regrowth. In general, these strategies are most successful when applied in a subacute or intermediate phase after brain injury such that the acute pathophysiological sequelae have played out and microenvironmental conditions have reached a relative equilibrium; therefore, implanted cells are not delivered into the "hostile" acute postinjury environment to succumb to the same factors driving degeneration of host cells/tissue. However, in

this phase, if larger tissue defects are being replaced, then mass transport becomes a paramount concern because nutrients and oxygen from host vasculature may not be available in the immediate proximity of implanted cells [44]. Moreover, across all phases of implant, the oxygen tension for cell/tissue growth in vitro, generally done under ambient conditions of 19%–20% oxygen, is considerably greater than the oxygen tension in the brain, which is typically 4%–6% [45,46]. This can cause a detrimental shock to implanted cells/tissue, although preconditioning the constructs via growth in oxygen-controlled chambers can at least partially mitigate this issue and may even induce greater functional benefits posttransplant [47–49].

As discussed above, given the limited endogenous potential for recovery in the brain, regenerative strategies must rely on externally derived solutions that either activate normally dormant regenerative processes or serve to directly reconstruct lost tissue and circuitry. A well-populated and growing set of strategies for restoring neural tissue involves the use of engineered scaffolds, defined as constructs designed to restore or replace compromised tissue through structural, trophic, and/or biological support. Biomaterial scaffolds—either acellular or cell-based—have been instrumental tools to advance research in these areas, providing the ability to manipulate host responses with a spatial and temporal specificity not possible with systemically or even locally administered agents. Such scaffolds have been developed to promote endogenous mechanisms of regeneration, elicit plasticity, drive neurogenesis, influence axonal guidance, and/or affect the generally inhibitory environment. To accomplish these goals, various biomaterial properties in these scaffolds are controlled and/or augmented, including physical properties (e.g., structure, porosity, geometry, microarchitecture), charge, adhesive mechanisms (e.g., addition of ligands), degradation mechanisms (e.g., time course, by-products), biological agents (e.g., growth factors, degradative enzymes, antiinflammatory compounds, genes, antibodies), or the addition of living cells. Years of research efforts have yielded a wide range of scaffolds that leverage one or more mechanism(s) of bioactivity (Table 34.1). These scaffolds are either acellular constructs with one or more bioactive agents incorporated into their structure (e.g., as coatings, or part of the base matrix) or constructs with living cells distributed throughout the scaffold either as dissociated suspensions or with an engineered architecture [115]. Both synthetic and natural materials have been used for regenerative scaffolds, with successful applications in clinical peripheral nerve repair as well as that of the spinal cord and dura mater (a protective layer of connective tissue surrounding the CNS) [15,50]. For brain tissue applications, scaffolds using synthetic materials, such as polyethylene glycol or poly ε-caprolactone, are generally fabricated as hydrogels—hydrophilic polymer networks with high (≥90%) water content—although fibrous electrospun scaffolds have also been used [50,51,70]. Certain scaffolds may combine synthetic and organic materials, either through blending of the base matrix materials into copolymers or composites (as with fibronectin-poly-*L*-lactic acid scaffolds) or synthetic hydrogels impregnated with bioactive proteins (e.g., neurotrophic factors) and/or living cells [50,51,116]. Several in vivo studies have shown that the inclusion of these organic components tends to promote better outcomes due to the presentation of naturally existing cues for cellular growth and attachment [51,71,117]. This chapter focuses primarily on biologically active scaffolds.

Table 34.1 Categories and examples of bioactive strategies for tissue-engineered scaffolds.

	Strategy	Example	References
Scaffold backbone	Extracellular matrix (ECM) components	Laminin, collagen, hyaluronic acid, fibronectin	[50–58]
		Decellularized tissue	[39,59–69]
	Non-ECM polymers	Methylcellulose, agarose, poly-L-lactic acid, tyrosine polycarbonate, silk, self-assembling peptides	[51,52,54,70–79]
Delivery of bioactive factors	Growth factors	NGF, CNTF, BDNF, FGF	[52,53,55,80–82]
	Matrix remodeling enzymes	Chondroitinase, phospholipase, hyaluronidase	[83–93]
Cell-based constructs	Dissociated cells (stem, primary, lines)	Neural stem/precursor cells Neurons Glial cells Dorsal root ganglia Marrow/mesenchymal stromal cells	[94–105]
	Tissue explants	Embryonic mesencephalic tissue Fetal cortical tissue	[106–108]
	Tissue-engineered living scaffolds for regeneration	Aligned astrocytes Aligned neurons/axons	[30,109–112]
	Tissue-engineered living scaffolds for direct cell/tissue replacement	Engineered neural networks	[112–114]

BDNF, brain-derived neurotrophic factor; *CNTF*, ciliary neurotrophic factor; *FGF*, fibroblast growth factor; *NGF*, neurotrophic growth factor.

34.5 Scaffold backbones

34.5.1 Extracellular matrix-based scaffolds

Many current scaffolds for brain reconstruction use natural materials to replace missing tissues. This strategy is designed to present a proregenerative environment through the presentation of biomolecules that already exist in the ECM. Many biomaterial scaffolds for the brain are hydrogels, either formed from natural polymers or containing one or more soluble factors [52,53]. These ECM materials are generally associated with neuronal growth and/or adhesion and are either used as an additive to a polymer (e.g., growth factor-laden hydrogels) or as the polymer itself, as with hyaluronic acid [39,54,55] (Fig. 34.2).

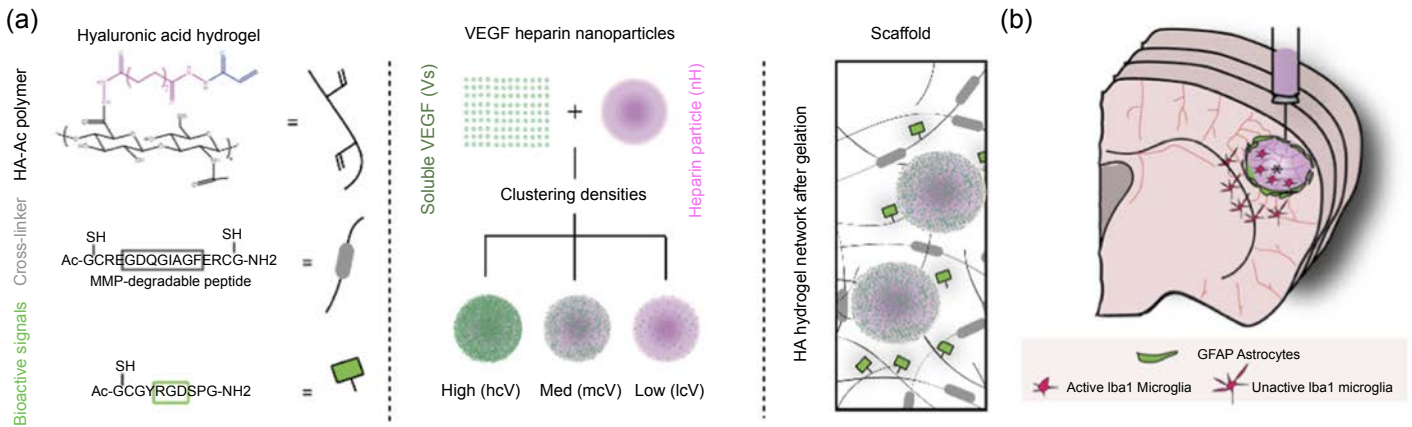

Figure 34.2 Injectable biomaterial hydrogels. (a) Schematic of an injectable hydrogel scaffold made of acrylated hyaluronic acid (HA) and carrying MMP degradable motifs, adhesion peptides, and heparin nanoparticles coated with varying densities of vascular endothelial growth factor (VEGF) to promote angiogenesis. (b) Schematic of the hydrogel being injected into a stroke site (*) in a mouse brain; native astrocytes and microglial cells are represented in and around the lesion.
Adapted with permission from L.R. Nih, S. Gojgini, S.T. Carmichael, T. Segura, Dual-function injectable angiogenic biomaterial for the repair of brain tissue following stroke, Nat Mater 17 (2018) 642–651. Copyright MacMillan Publishers Limited, Springer Nature.

In addition to their growth-promoting potential, these biomaterial scaffolds are inherently biocompatible; as naturally occurring materials, they are less likely to trigger an immune response, and their enzymatic/hydrolytic degradation does not yield any toxic by-products, as has been observed with some synthetic scaffolds [39]. In applications where a slowed degradation profile may be advantageous (e.g., for sustained therapeutic release of a growth factor), chemical modification methods (e.g., cross-linking) may modulate the biodegradability of natural materials; however, this may proportionally affect the extent of the modified polymer's immune response [53].

Biomaterial scaffolds benefit from the rich library of biomolecules present in the brain ECM. There is ample evidence that this natural scaffolding of glycoproteins, polysaccharides, and soluble cues is critical from early development to maturation of the brain. Early during embryogenesis, the ECM forms the functional framework of the brain as it modulates stem cell differentiation, directs neurons along different migratory paths, and guides axons to specific targets [56]. Postdevelopment, brain ECM serves as a reservoir for various signaling molecules and growth factors, as well as a structural support and organizational network for neurons and glia [15,56,57]. Several constituents of this matrix have been isolated and applied for use in tissue engineering due to their physical properties and biological effects, including collagen, laminin, hyaluronan, heparin, fibrin, fibronectin, and thrombospondin (Table 34.2). Although nonexhaustive, this table underscores the complexity of the milieu surrounding the neurons and axons of the brain.

34.5.2 Decellularized scaffolds

Another strategy for brain reconstruction builds scaffolds from tissue that has undergone decellularization (the removal of cellular material through mechanical dissociation, enzymes, and/or chemical agents), leaving primarily ECM constituents such as collagen, laminin, proteoglycans, and glycosaminoglycans (GAGs), and various growth factors [59,62,63]. Motivating this approach is the potential for the molecular composition of natural ECM to promote neuronal survival and/or growth while reducing the foreign body response compared with synthetic materials [63]. Ideally, the scaffolds would also provide cues for local cells to begin secreting ECM as part of the regenerative process [64]. Decellularized scaffolds have been created using both CNS (brain, optic nerve, spinal cord) and non-CNS (peripheral nerve, heart, muscle, liver, urinary bladder) tissue; depending on the source, decellularization, and assembly protocol, these scaffolds may also contain soluble factors or other small molecules [59,65,66]. On decellularization, the ECM may be processed into fibrous sheets, hydrogels, or an injectable liquid solution that self-assembles into a hydrogel on encountering physiological conditions (e.g., 37°C) [59,62]. For brain injury, the injectable solution may be more generalizable, as it provides a means to minimize the invasiveness of injection and fill large and/or irregular voids in tissue [52,64].

Broadly, characterization studies for decellularized scaffolds involve determining the scaffold purity (i.e., the amount of residual cellular material, proteolytic

Table 34.2 Various extracellular matrix constituents/families that have been used in tissue-engineered scaffolds and a subset of their associated functions.

	Collagen	**Laminin**	**Hyaluronan**	**Heparin**	**Fibrin**	**Fibronectin**	**Thrombospondin**
Type	**P**	**P**	**GAG**	**GAG**	**P**	**Gp**	**Gp**
Migration	✓	✓				✓	
Structure	✓		✓				
Neurite growth	✓	✓		✓	✓		✓
Development/ Differentiation	✓	✓		✓	✓		✓
Adhesion	✓	✓	✓		✓	✓	
Wound healing	✓		✓	✓		✓	

GAG, glycosaminoglycan; *GP*, glycoprotein; *P*, protein.

enzymes, or other contaminants) and/or investigating its effects on cell behavior in vitro. Several in vitro studies suggest that certain effects of decellularized scaffolds depend on the source tissue used; namely, that tissue-specific ECM compositions may direct stem cell differentiation into cell types associated with the source tissue [63]. Along these lines, comparisons of CNS-derived versus non-CNS-derived scaffolds have shown that CNS scaffolds supports greater neuronal differentiation and increased neurite growth, although some neuronal differentiation still occurs on non-CNS scaffolds [64,67–69]. In vivo, decellularized scaffolds derived from both brain and urinary bladder tissue have been implanted in animal TBI and stroke models as injectable liquid hydrogels [62,64]. These studies have shown that decellularized scaffolds can reduce lesion volume and both short- and long-term degeneration following injury [60,64,69]. At least one study reported improved behavioral outcomes in a mouse TBI model, although the scaffold was mixed with neural progenitors, which may have played an additional role in functional recovery [61]. These injectable scaffolds also elicited infiltration by astrocytes, microglia/macrophages, and neural progenitors; however, there has been no evidence that these scaffolds enable the generation of new functional neuronal tissue [69,99].

The advantages of these tissue-derived scaffolds are highly dependent on the decellularization protocol. Current methods inherently eliminate the native architecture and organization of the native ECM. The spatial organization of the various proteins, GAGs, and other biomolecules within native ECM is the framework for their interactions and downstream cellular effects, but the extent to which this loss affects the functional utility of decellularized scaffolds has yet to be fully determined [63]. Furthermore, significant changes to the ECM composition (e.g., through the loss of signaling molecules or growth-promoting proteins) may reduce tissue-specific advantages of the ECM source [64]. Variations in the decellularization process may leave residual cellular debris, lipids, DNA, or other immunogenic material [63,67]. The purity of the decellularized scaffold thus influences the severity and longevity of the inflammatory response, as well as the extent of tissue regeneration postimplant [67]. The current lack of a set of standards for tissue decellularization, postprocessing verification of purity, and incomplete understanding of how the various elements within the final scaffold interact with wound healing and the foreign body response in vivo exacerbate these challenges [63]. As they are generally rich in collagen, decellularized scaffold hydrogels tend to degrade quickly on delivery into the brain, which may also negatively impact the neuroprotective and/or regenerative capabilities [64]. Covalent cross-linking of the scaffold before implant may slow its degradation at the risk of eliciting or worsening a chronic foreign body response; as such, further work must be done to determine the optimal scaffold lifetime.

34.5.3 Non-ECM polymer-based scaffolds

Polymeric scaffolds for brain regeneration may also be made from materials not normally found in brain ECM. In particular, polymers that form gels at

physiological conditions are valuable due to the often irregularly shaped, anisotropic cavities that form following brain injury; such hydrogels may be microinjected as liquids (i.e., with minimal invasiveness) and conform to the cavity as needed [75,76]. Non-ECM polymers include methylcellulose, agarose, and various methacrylates (poly(2-hydroxyethyl methacrylate), poly(hydroxypropyl methacrylate)), which have been used in animal TBI and other CNS lesion models [54,76–78,116,118,119]. For many such studies, these materials are functionalized through immobilization or bonding of biomolecules to facilitate more biofidelic tissue/scaffold interactions, promote host cell growth, adhesion, and survival, and/or attenuate the inflammatory response following injury [76,119]. Examples include the covalent bonding of laminin to methylcellulose gels, copolymers of hyaluronic acid and poly-D-lysine, and "hybrid" scaffolds composed of electrospun poly-L-lactic acid + fibronectin fibers embedded in an agarose/methylcellulose hydrogel [76,116,120]. Other synthetic materials being developed for brain reconstruction include 3D-printed polyurethane hydrogels seeded with neural stem cells, with functional motor recovery in a zebrafish TBI model [121]. Cell-seeded scaffolds of electrospun tyrosine-derived polycarbonate have also been implanted in mouse models, with the implanted cells surviving for at least 3 weeks [100]. Although historically used for degradable sutures, silk fibroin has more recently been processed into self-assembling hydrogels for use in animal stroke models [74] (Fig. 34.3). These silk-based gels have primarily been used to fill lesions, although at least one study has shown that silk scaffolds are capable of delivering mesenchymal stem cells to the injury site with evidence of subsequent functional recovery [74,122].

A class of non-ECM materials called self-assembling peptides may also serve as scaffolds for brain repair; on introduction to various salts at physiological conditions such as cerebrospinal fluid, these peptides spontaneously form hydrogels composed of networks of nanoscale fibers (SAPNs) [79,123,124]. The nanoscale topology of SAPNs permits direct interactions with ECM components, while their structure may be modified to include biological motifs or carry bioactive payloads (e.g., drugs, cells). These peptide scaffolds have been shown to enable cell attachment and growth in vitro as well as cell infiltration, axon regeneration, and reduced CNS lesion size in rat, zebrafish, and hamster models [72,73,79] (Fig. 34.4). One such material, RADA16, forms a nanofibrous hydrogel in vivo and is one of the most widely investigated SAPNs due to its hemostatic and regenerative properties [124]. In a zebrafish midbrain injury model, the introduction of a RADA16 scaffold acutely postinjury resulted in angiogenesis and the formation of mature neurons around the lesion site [75]. Similarly to other synthetic scaffolds, functional recovery increased when the RADA16 scaffold was functionalized with a bioactive motif [75]. Notably, RADA16 scaffolds have induced enough axonal regeneration to enable functional recovery in at least one mammalian optic nerve model [79] (Fig. 34.4).

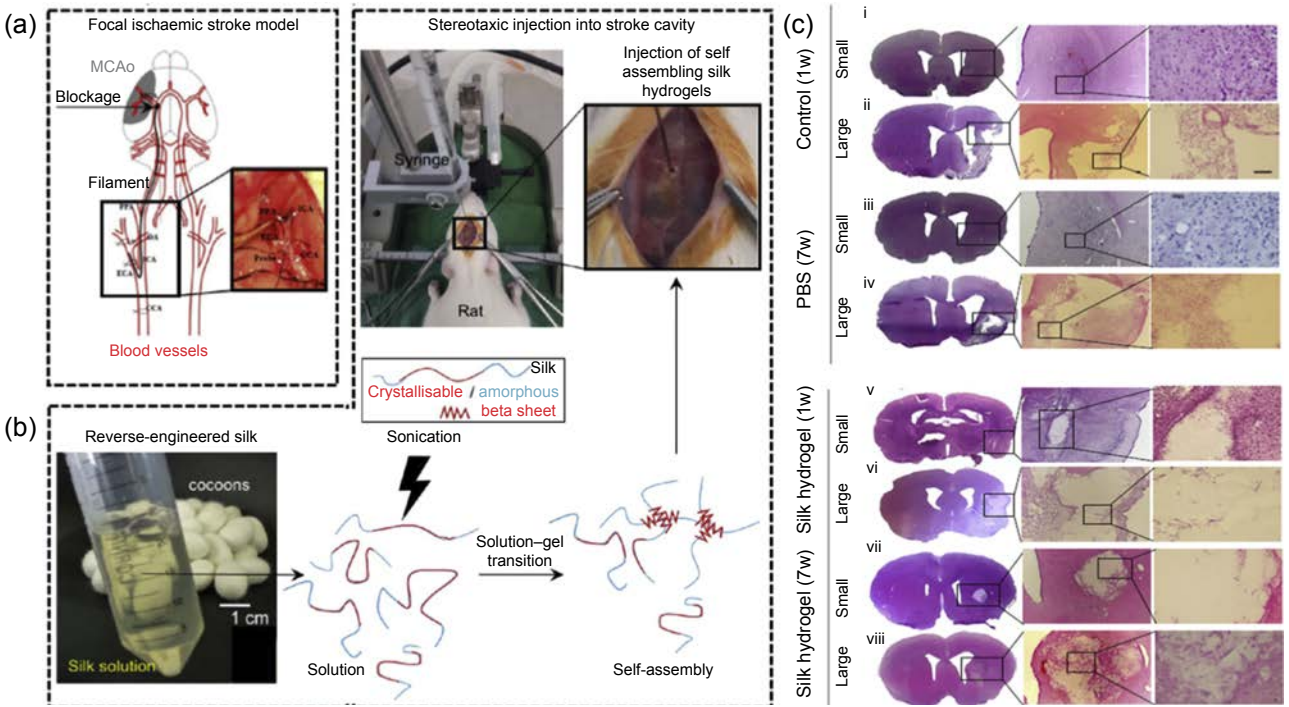

Figure 34.3 Self-assembling silk hydrogels. Preparation and administration of self-assembling silk fibroin hydrogels into rats following focal cerebral ischemic stroke. (a) Diagram of the stroke injury model, defined as a right transient middle cerebral artery occlusion (MCAo), before injection of self-assembling silk fibroin hydrogel into the lesion site. (b) 4% w/v silk fibroin was harvested from *Bombyx mori* cocoons and sonicated to initiate a solution–gel transition (i.e., self-assembly) immediately before injection. (c) Representative hematoxylin- and eosin-stained coronal brain sections from animals given (v–viii) self-assembling silk fibroin hydrogels, (iii, iv) PBS only, or (i, ii) no-injection (control) at 1 or 7 weeks. Self-assembling silk fibroin hydrogels exhibited good space conformity and retention in both small and large stroke cavities.
(a) MCAo image photograph and diagram reproduced with permission from S. Lee, M. Lee, Y. Hong, J. Won, Y. Lee, S.G. Kang, K.T. Chang, Y. Hong. Middle cerebral artery occlusion methods in rat versus mouse models of transient focal cerebral ischemic stroke. *Neural Regen Res* **9**, 2014, 757-8. Copyright Neural Regeneration Research, Wolters Kluwer Medknow Publications. Adapted with permission from N. Gorenkova, I. Osama, F.P. Seib, H.V.O. Carswell, In vivo evaluation of engineered self-assembling silk fibroin hydrogels after intracerebral injection in a rat stroke model, ACS Biomater Sci Eng (2018). https:doi.org/10.1021/acsbiomaterials.8b01024. Copyright American Chemical Society.

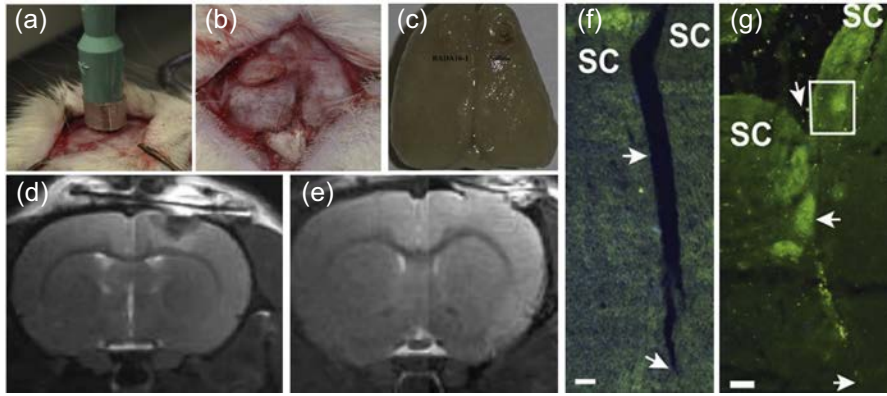

Figure 34.4 Self-assembled peptide nanofibers (SAPNs). (a and b) RADA16-I-based SAPNs in brain repair in a cortical resection model in adult rats. (c) Macroscopic appearance after treatment with RADA16-I (left hemisphere) and saline (right hemisphere). (d and e) MRI study showing the area of T2-hyperintense around the lesion site in the right frontal lobe after treatment with (d) electrocautery and (e) RADA16-I. SAPNs allow axons to regenerate through the lesion site in brain. The dark-field composite photos are parasagittal sections from animals 30 days after lesion and treatment. (f and g) RADA16-I-based SAPNs in axon regeneration following an optic tract transection in hamsters. (f) Section from brain of a 30-day-old hamster with 10 μL of saline injected in the lesion at postnatal day 2 (P2). The retinal projections, in light green at the top left edge of the cavity, have stopped and did not cross the lesion. Arrows indicate path and extent of knife cut. (g) A similar section from a 30-day-old hamster with a P2 lesion injected with 10 μL of 1% SAPNs. The site of the lesion has healed, and axons have grown through the treated area and reached the caudal part of the superior colliculus (SC). Axons from the retina are indicated by light-green fluorescence. The boxed area is an area of dense termination of axons that have crossed the lesion. *Arrows* indicate path and extent of knife cut. Scale bars: 100 μm.
Adapted with permission from G.K.K. Leung, Y.C. Wang, W. Wu, Peptide nanofiber scaffold for brain tissue reconstruction, Methods Enzymol 508 (2012) (Elsevier Inc.). Copyright Elsevier. Adapted with permission from R.G. Ellis-Behnke, Y.X. Liang, S.W. You, D.K.C. Tay, S. Zhang, K.F. So, G.E. Schneider, Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision, Proc Natl Acad Sci USA 103 (2006) 5054–5059. Copyright Proceedings of National Academy of Sciences.

34.6 Scaffolds as vehicles for delivery of bioactive factors

In addition to these natural matrix/scaffolding materials, biomaterial scaffolds may contain soluble factors to limit neuronal attrition and/or promote cell migration or axon outgrowth after injury. Neurotrophins such as brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophic growth factor (NGF) have been shown to promote neuronal regeneration and axonal growth when injected into peripheral nerve and spinal cord lesions [80,81,125]. Neurotrophin delivery via scaffolds has been explored for spinal cord injury models, with demonstrated regeneration and functional

recovery [126,127]. Other factors or cytokines may be chosen for their potential to modulate the behavior of endogenous neural stem cells/neural progenitor cells (NSCs/NPCs); one such cue, BDNF, has been associated with NSC/NPC survival, migration, and differentiation [55]. NSC/NPC-focused approaches using hydrogels have, to date, yielded NSC/NPC survival and proliferation, although they are limited by the rapid degradation of the hydrogel [55].

34.7 Cell-based scaffolds

34.7.1 Dissociated cells

In addition to limiting the damage to existing neurons, scaffolds may be engineered to deliver cells to replace those lost during injury. Scaffold-based cell delivery has been explored predominantly for potential stroke or TBI therapies in animal models [53]. Most approaches use NSCs/NPCs, although the use of mesenchymal stromal cells (MSCs) has also been explored in rodent stroke models [55]. Interestingly, although transplanted MSCs have not differentiated into neural cells, they have yielded diminished glial scarring, increased angiogenesis and axonal sprouting at the lesion site, and functional recovery out to at least 4 months [94,95]. The existence of established differentiation protocols for differentiating induced pluripotent stem cells into various types of neuronal precursors makes them attractive sources for cell transplants. However, the risk of tumor formation should be considered for various induced stem cell sources. Beyond physical cell replacement, cell-based approaches offer the potential to reestablish damaged neural connections through synaptic integration [62,99]. One cell-based approach generates pathways for targeted axonal outgrowth from transplanted cells in vivo through virally induced expression of fibroblast growth factor and NGF; this strategy has successfully induced axonal growth from transplanted dorsal root ganglia across lesions in adult rat brains [101,128].

The efficacy of cell-based scaffolds is primarily dependent on the survival of the transplanted cells, with scaffold cell survival positively correlated with functional outcomes [96,106]. However, because of the brain's natural response to injury, the environment where the cells are most needed is detrimental to their survival. Postinjury, the affected area is flooded with proinflammatory factors, active immune cells, and a lack of trophic support which have, to date, resulted in heavy attrition of transplanted cells; various research groups have reported cellular losses of 68%–99% [53,55,97]. Any subsequent immune reaction caused by the scaffold or the cells themselves further limits their potential for brain reconstruction. This attrition prevents most cell-based scaffolds from surviving long enough to rebuild lost connections and enable functional recovery [55,102]. To address these challenges and facilitate cell survival, many cell-based scaffolds are being designed with one or more biomolecules to protect the cells from neurotoxic phenomena, reduce the severity of inflammation, and/or provide the trophic support needed for angiogenesis, neuronal differentiation, growth, and adhesion [97,103]. Direct comparisons of stem cell viability with and without supporting scaffolds have shown that the scaffolds significantly improve survival [103]. Other studies in rat and mouse models have found that the incorporation of stem cells

in decellularized or biomaterial scaffolds reduced the extent of injury while improving motor and cognitive function post-TBI [96,104,129]. Optimization of specific surgical strategies, such as the timing of delivery postinjury and the proximity of the scaffold relative to the injury, may also have positive effects on cell survival [105,130].

34.7.2 Tissue explants

Rodent studies have demonstrated that fetal CNS tissue explants post-TBI decrease glial scarring, improve behavioral outcomes, and integrate with host tissue [82,96]. Similarly, clinical studies in PD patients showed that fetal tissue transplants containing dopaminergic neurons elicit lasting motor improvement with better performance than was possible with medication pretransplant [96,106–108]. However, the observed therapeutic benefits of tissue transplants in the brain are limited by the practicality of sourcing tissue.

34.7.3 Tissue engineered living scaffolds

The functional recovery of brain tissue requires both reconstitution of network architecture (i.e., microarchitecture, long axonal tracts) and regeneration/replacement of multiple cell types. These requirements are the motivation for living scaffolds—tissue-engineered constructs with one or more living cell populations supported by one or more biomaterials with a defined 3D architecture [112,113]. Living scaffolds may recapitulate multicellular compositions and complex microarchitectures through tailoring of their physical (e.g., stiffness, porosity, anisotropy) and/or cellular (cell density, phenotype(s)) properties to provide structural parity and cellular integration with the tissues of interest. Additional factors or molecules for signaling, growth, and similar outcomes may also be incorporated into the encapsulating biomaterial scaffold [115,131].

For one such strategy, we use discrete populations of neurons connected by long axonal tracts to mimic the long-distance axonal pathways that may be lost to injury or disease [111]. These living constructs are encased within soft hydrogel microcylinders, with an ECM substrate of collagen and laminin supporting axonal growth through the lumen [111,112,114]. We have referred to these constructs as "micro-tissue-engineered neural networks (micro-TENNs)," and they were developed to reconstruct lost axonal circuitry through microinjection delivery and synaptic integration with host neurons, providing a preformed axonal tract rather than needing to induce long-distance, targeted axonal growth in vivo [112] (Fig. 34.5). To date, micro-TENNs seeded with embryonic cortical neurons have been shown to survive for at least 1 month posttransplant in a naïve rat model, with evidence of neurite growth and synaptic formation with the host [113]. Similarly, micro-TENNs with dopaminergic neurons have also been developed as a potential treatment for PD by spanning the nigrostriatal tract and restoring dopaminergic inputs to the striatum; these constructs have survived for at least 1 month with dopaminergic axons spanning the nigrostriatal pathway [132].

Living scaffolds may also be designed to recruit endogenous regenerative mechanisms. For instance, the rostral migratory stream is characterized by a tube of aligned astrocytes that guides NPCs from the SVZ to the olfactory bulb. We developed a technique to direct astrocytes to self-assemble into longitudinally aligned networks within hydrogel-collagen

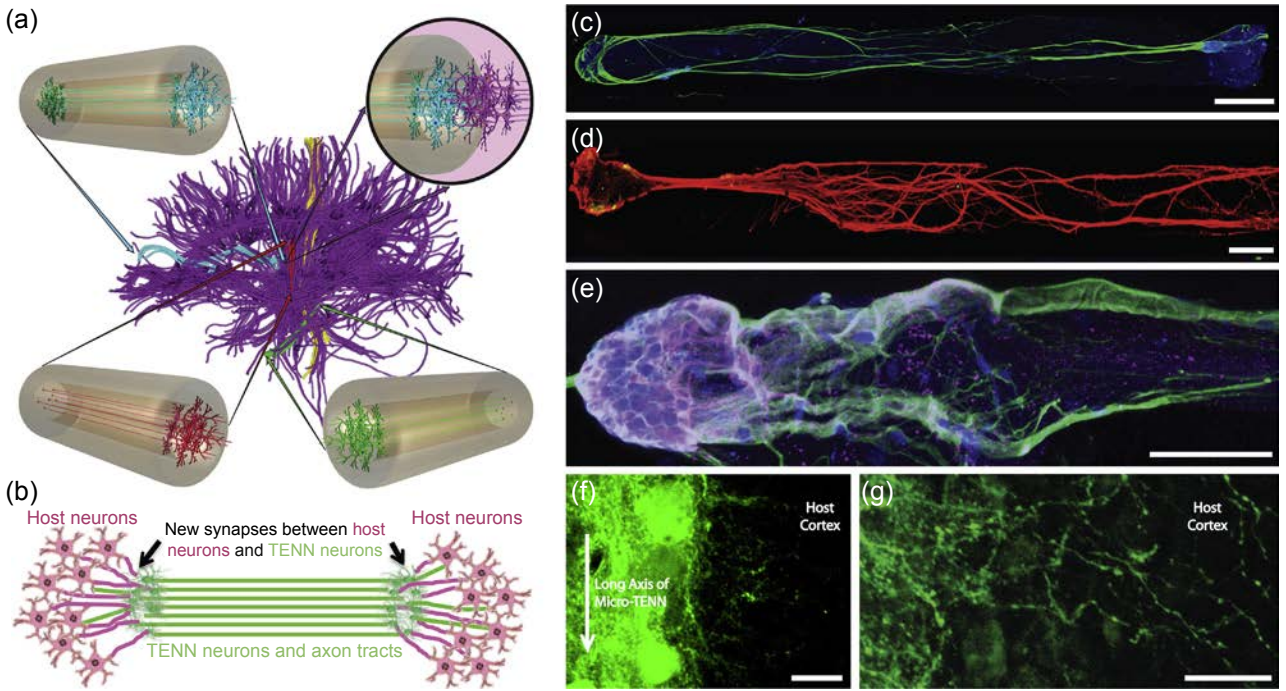

Figure 34.5 Microtissue-engineered neural networks (micro-TENNs). (a) Diffusion tensor imaging representation of the human brain as a network of functionally distinct areas connected by long axonal tracts (blue: corticothalamic pathway, red: nigrostriatal pathway, green: entorhinal–hippocampal pathway). Micro-TENNs may be grown and implanted to reconstruct these tracts following brain injury. (b) Conceptual schematic of a micro-TENN (green) forming synapses with two host neuronal populations (pink) as a means to replace lost or damaged pathways. (c) Confocal reconstruction of a bidirectional micro-TENN immunolabeled for axons (β-tubulin, green) and cell nuclei (Hoechst, blue). (d) Confocal reconstruction of a unidirectional micro-TENN immunolabeled for axons (tau, red) and neuronal somata/dendrites (MAP2, green). (e) Confocal reconstruction of a unidirectional micro-TENN immunolabeled for axons (β-tubulin, green), cell nuclei (Hoechst, blue), and neuronal somata/dendrites (MAP2, purple). (f and g) Confocal reconstruction of a GFP^+ micro-TENN posttransplant in rat cortex, with micro-TENN axons growing into host cortex. Adapted with permissions from Wolters Kluwer Medknow Publications.

microcylinders, mimicking the rostral migratory stream as a potential guide for NPCs toward injury sites [30,109,110]. This "tissue-engineered rostral migratory stream" has been shown to induce neuronal migration along the astrocyte "cables" in vitro, although their ability to redirect NPCs in vivo must still be determined [30]. Similar approaches may leverage developmental phenomena such as axon pathfinding, where pioneer axons serve as a living scaffold to guide the growth of axonal growth cones via both haptotactic and chemotactic cues [112]. Here, preformed constructs comprising long, living axon tracts effectively serve as tissue-engineered pioneer axons to guide regenerating axons based on the newfound mechanism of axon-facilitated axon regeneration [111].

34.8 Conclusion

The brain is the most complex organ in the human body, and the exquisite architecture and connectivity must be appreciated if sophisticated biomaterial and/or tissue engineering strategies are to elicit functionally meaningful regeneration and reconstruction. This chapter described the state of the art for emerging strategies to facilitate regeneration, reconnectivity, and/or reconstruction of exquisite neuronal–axonal–glial networks following degeneration caused by brain injury or neurodegenerative disease. In general, we discussed biomaterial/biological scaffolds aimed at filling defects, providing structural support, providing guidance cues (generally anisotropic), delivering biological agents, and/or providing preorganized cells. These strategies were organized as organic/biologically derived scaffolds (e.g., ECM-based scaffolds), nonorganic polymer-based scaffolds (e.g., hydrogels), and so-called "living scaffolds" (e.g., anisotropic cell-laden scaffolds).

Biomaterial scaffolds have demonstrated utility for 3D structural support, as well as the introduction of chemotactic cues or drugs to reduce inflammation, improve neuronal survival following focal injury, and promote axon outgrowth. In parallel, cell-based scaffolds have been shown to improve behavioral outcomes relative to acellular materials, although the mechanisms underlying these benefits are still under investigation. The goal of comprehensive brain tissue reconstruction, especially for large lesions, is unlikely to be met by one-dimensional strategies such as a single biomaterial scaffold or suspensions of single cells. Rather, multifaceted strategies harnessing state of the art in 3D biomaterials, stem cell biology, drug delivery, and tissue engineering are likely needed to address the formidable challenges. Indeed, although neuronal integration has been reported for some for cell-based therapies alone, the formation of new tissue likely requires a complex composition of living cells and ECM with a level of organization that has not yet been realized outside of tissue explants [102]. The engineering of tissue analogues, or living scaffolds, more closely approximates these features by combining the advantages of biomaterials and cell replacement; as such, future strategies for brain reconstruction may likely resemble engineered tissue explants for axonal guidance, replacement, or stem cell recruitment. Collectively, these emerging strategies are working toward the ultimate goal of preventing cell/axonal loss, promoting healing, reconstructing lost neural architecture/connections, and ultimately facilitating functional recovery following nervous system disorders.

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