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A Roadmap of Peptide-Based Materials in Neural Regeneration

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Injuries to the nervous system lead to irreversible damage and limited functional recovery. The peripheral nervous system (PNS) can self-regenerate to some extent for short nerve gaps. In contrast, the central nervous system (CNS) has an intrinsic limitation to self-repair owing to its convoluted neural microenvironment and inhibitory response. The primary phase of CNS injury, happening within 48 h, results from external impacts like mechanical stress. Afterward, the secondary phase of the injury occurs, originating from neuronal excitotoxicity, mitochondrial dysfunction, and neuroinflammation. No golden standard to treat injured neurons exists, and conventional medicine serves only as a protective approach to alleviating the symptoms of chronic injury. Synthetic peptides provide a promising approach for neural repair, either as soluble drugs or by using their intrinsic self-assembly propensity to serve as an extracellular matrix (ECM) mimic for cell adhesion and to incorporate bioactive epitopes. In this review, an overview of nerve injury models, common in vitro models, and peptide-based therapeutics such as ECM mimics is provided. Due to the complexity of treating neuronal injuries, a multidisciplinary collaboration between biologists, physicians, and material scientists is paramount. Together, scientists with complementary expertise will be required to formulate future therapeutic approaches for clinical use.

1. Introduction

1.1. Peripheral Nerve Injury (PNI)

The peripheral nerve is made up of axons derived from neurons, Schwann cells (SCs), connective tissues, and vascular networks.[1–6] External impact, surgical procedures, and congenital defects are the most common causes of PNI. In brief, PNI is classified into 3 types: neurapraxia with blockage of nerve conduction, axonotmesis with complete axonal disconnection, and neurotmesis with complete transection of the peripheral nerve.[5] The mechanisms of injury include axonal swelling, myelin disintegration, and axon degeneration at the distal end of axons, while the immune response and scar formation are mainly restricted to the proximal end of axons.[6]

Understanding the natural repair mechanisms of PNI is crucial for PNS regeneration. After injury, 2 processes are induced: axonal degeneration and clearance of myelin debris. Surviving cells secrete proinflammatory cytokines to recruit macrophages to clear out myelin debris, allowing for remodeling of the injured site, and stimulating SCs to secrete neurotrophic growth factors to boost axonal regeneration.[1] In the clinic, end-to-end suturing of nerve stumps is the preferred treatment for short nerve gaps (less than 1 cm), while autografts (derived from oneself) are considered the standard treatment for bridging long nerve gaps. However, issues that may arise for autografts include limited availability of donor tissue, dimensional mismatch between the graft and native nerve, and potential sequelae from additional surgery of the donor nerve.[7] Alternative approaches include allografts (derived from the same species) and xenografts (derived from other species), but they are bound to face the tedious process of immunosuppression or decellularization.[1] Synthetic nerve grafts have the potential to overcome these hurdles; however, the design of nerve grafts must consider their biocompatibility, biodegradability, and proper mechanics to mimic the nature of peripheral nerves.[6]

1.2. Central Nerve Injury

1.2.1. Spinal Cord Injury (SCI)

The spinal cord, connecting the brain and peripheral nerve through dorsal and ventral roots, is a long cylindrical nerve

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bundle surrounded by intervertebral discs, blood vessels, muscles, and ligaments. Each bundle contains gray matter, including soma and dendrites, surrounded by white matter that is composed of axons and various neuroglial cells.[1,3] Astrocytes have star-shaped morphology and maintain the homeostasis of the spinal cord. Oligodendrocytes are the myelin-producing cells of the CNS, and convolve the axons, enabling more efficient transduction of electrical signals along neurons. Microglia, the immune cells of the spinal cord, regulate the microenvironment by responding to pathogens and removing cellular debris. Ependymal cells serve as the central canal of the spinal cord and maintain the cerebrospinal fluid. Pericytes within the basement membrane are crucial for angiogenesis and maintenance of the blood-spinal cord barrier (BSCB).[1,8]

The pathophysiology of SCI is composed of 2 phases. The primary injury typically results from mechanical impacts on the spinal cord that damage the neurons, glia cells, and microvasculature. Acute symptoms, such as tissue damage, neuronal loss, and vascular rupture, further trigger the secondary injury cascades of biochemical and cellular responses. The secondary injury phase, occurring over a few days to several months, includes hypoxia and ischemia of the injury site, glial scar formation, and dysregulation of neurotransmitters.[3,9] The glial scar is beneficial in preventing further damage and facilitating blood circulation at the injury site. However, scar tissues, which mainly consist of inhibitory ECM components such as chondroitin sulfate proteoglycans (CSPGs), impede SCI regeneration because the thick layer of tissues hinders axon regrowth.[6] Additionally, myelin-associated inhibitory molecules, such as myelin-associated glycoprotein (MAG), Nogo-A, etc., exacerbate the difficulty of SCI repair.[10]

In the clinic, neuroprotective and anti-inflammatory drugs are the principal treatment for patients in the first phase of SCI. Surgical procedures to remove bone and disc fragments and blood pressure stabilizing treatment help prevent further damage at the injury site. Drug delivery, however, has limited efficacy due to the restricted transport across the BSCB. After all, the aforementioned treatments are primarily designed to alleviate trauma, not to promote spinal cord regeneration.[3,9] Several studies indicate that cellular therapies, such as neural stem cells (NSCs) and mesenchymal stem cells (MSCs), open up new opportunities in SCI repair. However, there are some drawbacks to cellular therapy, including low cell survival, difficult standardization due to various cell sources, uncertainty of cellular behavior, and lack of physical guidance for axonal growth.[3,9] The pros and cons of nerve grafts for SCI repair are similar to those of PNI, which are described in the previous section (1.1). Therefore, it is of great interest to develop biomaterials with suitable physical properties (e.g., guidance, stiffness, etc.) and appropriate biochemical properties (e.g., bioactive, anti-inflammatory, etc.). Other important considerations are facile handling and applications for minimally invasive surgery, allowing physicians to use the biomaterials with ease in the clinic.[1] In addition, electrical stimulation and brain-spine interfaces successfully enhanced locomotor activity in spinal cord injury patients.[11] In the future, treatment options combining biomaterials with neuronal stimulation devices might be a strategy to overcome the burdens of SCI.

1.2.2. Traumatic Brain Injury (TBI)

TBI is amongst the most frequent causes of CNS injury and the leading cause of mortality in young adults worldwide.[12,13] The primary phase of TBI occurs when the head receives external mechanical insults. Common acute symptoms are contusion, hematoma, hemorrhage, and axonal injury.[4,14] These neurovascular impairments lead to secondary injuries at the cellular level, including excitotoxicity, mitochondrial dysfunction, oxidative stress, neuroinflammation, axonal degeneration, and apoptotic cell death.[4,14,15] Likewise, reactive microglial cells and astrocytes secrete CSPGs and other myelin-associated inhibitors to form glial scars to prevent further secondary injury, which limits the self-repair capacity after TBI.[4]

Currently, therapeutic treatment aims to stabilize the primary lesion and prevent it from deteriorating into the secondary injury. In the clinic, surgical intervention helps reduce intracranial pressure and edema. Ng et al. summarized abundant research for clinical trials devoted to the mitigation of the secondary injury of TBI, including the pathophysiology, therapeutic targets, and their possible corresponding therapies.[15] However, the blood-brain barrier (BBB), which protects and regulates the immune responses of the brain, also limits the delivery efficiency of external substances.[16] Aertker et al. note that cellular therapy, like NSCs, MSCs, adipose stromal cells, etc., can modulate the inflammatory response and secrete neurotrophic factors. Nonetheless, continuous administration of cells and non-autologous cell sources will inevitably lead to immunogenic problems.[4] Some clinical studies showed minor neurological improvement in a few patients without adverse effects. More in-depth research and clinical trials are needed to address these challenges.[4,15] Establishing more specific clinical analytical methods, imaging tools, and biomarkers will further aid pharmaceutical development.[4] Furthermore, injectable materials or customized nanocarriers that cross the BBB pave the ways for new therapeutic options for TBI patients.

2. Experimental Models to Study CNS and PNS Injuries

2.1. In Vitro Models

This is an interdisciplinary review for non-neurologists aiming to contribute to the field of neural repair. Thus, it is critical to note that there is a wide variety of neurons, e.g. sensory neurons, interneurons, projection neurons, excitatory neurons, and inhibitory interneurons, classified based on diverse molecular, morphological, connectional, and functional properties.[17] Therefore, the following sections briefly introduce some characteristics of neural cell lines, primary cells, and stem cells as well as an ex vivo model that is often used to study novel treatment approaches for nerve injuries.

2.1.1. Cell Lines

Cell lines are preferably used over primary cells for initial studies because of their advantages of commercial availability, rapid growth, and large number of similar cells.[18] In general, positive results from cell lines are encouraging and should be further

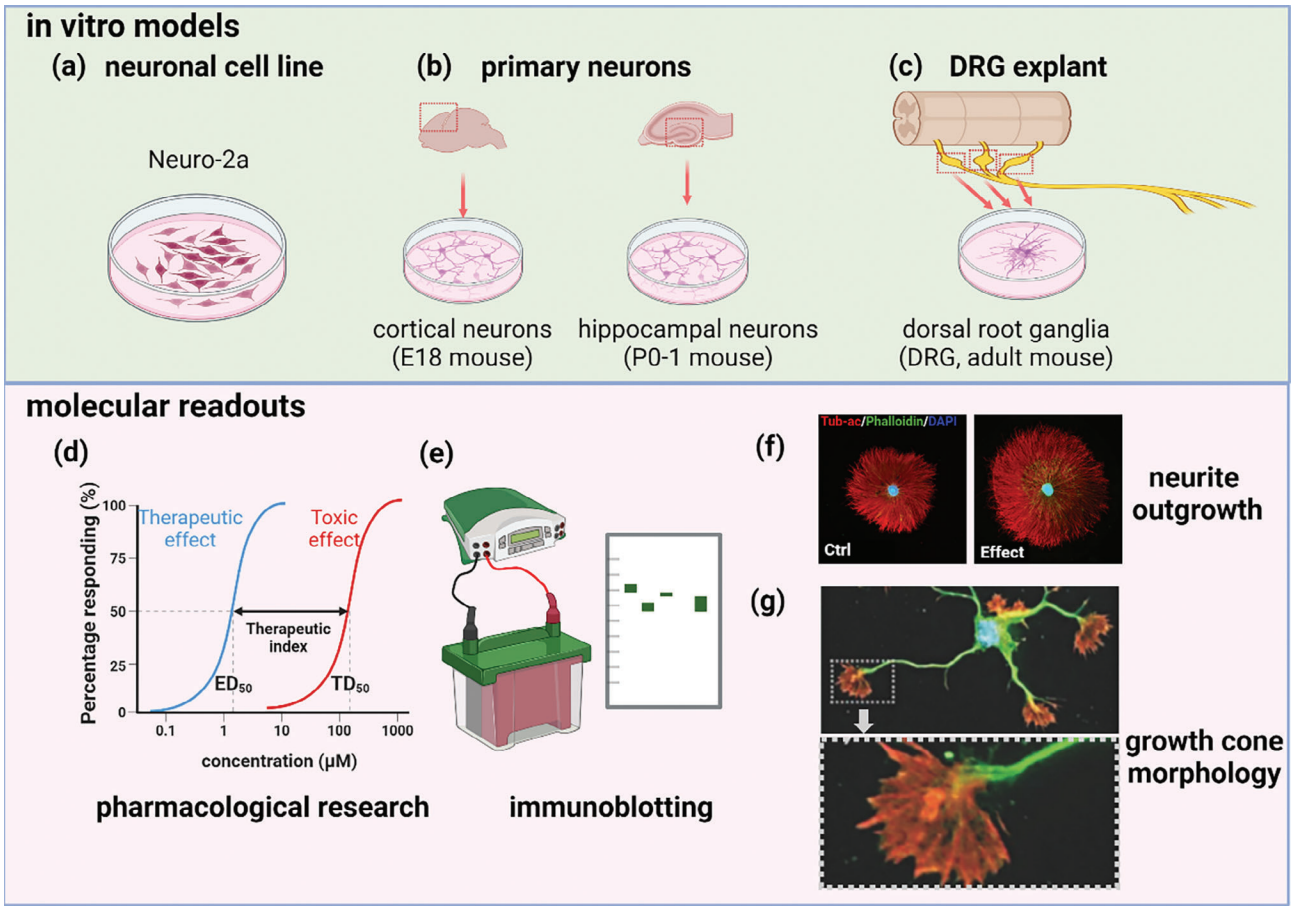

Figure 1. Examples of common in vitro models for neuroscience (generated using Biorender). a) cell Neuro2a cells are one of the commonly used neuronal cell lines in neuroscience research to extract RNA/ protein for further analysis. b) Primary cells: there are standard protocols for hippocampal and cortical neuron culture and they can form complex structures in vitro. c) Dorsal root ganglia (DRG) explants are commonly employed in neurite growth research. d) A schematic of pharmacological research depicting a typical and preliminary readout to test the therapeutic window of a compound on neuronal cell lines. e) An overview of the immunoblot setup and readout that apply to in vitro models to understand protein expression of neuronal cells after being treated with compounds. f) Neurite outgrowth assay using DRG explants to quantify, e.g., neurite area. The control group was DRGs cultured alone in the medium and the experimental group was DRGs cultured with repair phenotype SCs. Adapted with permission.[42] Copyright 2023, EMBO press. g) Immunostaining of 2 neuronal markers, Phalloidin and Tuj1, showing the morphology of growth cones from mouse primary hippocampal neurons that were cultured on peptide-polydopamine nanofibers. Adapted with permission.[41] Copyright 2018, WILEY-VCH GmbH.

supported by evidence from further in vitro and/or in vivo injury models. The drawbacks of cell lines are their chromosomal instability and phenotypic variation in relation to neurons in vivo.[18] The following section highlights some commonly used neural cell lines.

PC12 cell line (ATCC CRL-2266) is derived from rat adrenal pheochromocytoma cells, a type of neuroendocrine tumor growing from chromaffin cells.[19] When treated with nerve growth factor (NGF), PC12 cells differentiate into neuron-like cells with similar morphology and physiological functions, including acetylcholine secretion and neurite formation.[19,20] This cell line has the advantage of a short differentiating time and serves as a model for neural secretory cells.[20,21]

Neuro2a (ATCC CRL-131), also known as N2a, is a cell line isolated from mouse brain tissue, depicted in **Figure 1a**. The common method to induce N2a differentiation is by withdrawing serum. With neuronal and amoeboid stem cell morphology, differentiated N2a cells highly express neurofilaments, which is suitable for axonal outgrowth research.[22] Viability assays (e.g., MTT assay and LDH assay) and apoptosis assays (e.g., TUNEL assay) are employed to measure cell survival and apoptosis.

SH-SY5Y cell line (ATCC CRL-2266) originates from human neuroblastoma cells.[23] This cell line has 2 different phenotypes: which are neuroblast-like (N-type) and epithelial-like (S-type). The N-type of SH-SY5Y cells can further differentiate into a more mature neuronal phenotype, express neural markers, and synthesize neurotransmitters. On the other hand, the undifferentiated cells express immature neural markers.[24] The advantages of SH-SY5Y cell culture over other neural cell lines are its human origin and various differentiation protocols.[23–25]

2.1.2. Stem Cells

Stem cells bear the potential of self-renewal and differentiation into different cell types. Due to their differentiation potential,

stem cells can be classified into totipotent stem cells, pluripotent stem cells (PSCs), multipotent stem cells (MSCs), and unipotent stem cells.[26,27] For instance, stem cells are able to generate pure glutamatergic neurons or can be differentiated into motor neurons.[28]

PSCs are easily cultured in vitro and can be differentiated into most cell types.[29] They can be obtained from pre-implantation human embryos or from somatic cells that are reprogrammed by specific transcription factors into a pluripotent state, also known as induced pluripotent stem cells (iPSCs).[27,29] iPSCs can be derived from sources other than embryos of the same species, circumventing the associated ethical and immunogenic concerns associated with using embryos. The advancement and technological details of iPSCs are summarized elsewhere.[27,29,30] Furthermore, scientists have applied iPSC-derived neurons to investigate neural regeneration such as SCI.[29–31]

MSCs are also referred to as mesenchymal stem cells or multipotential stromal cells.[32] These stem cells can differentiate into specific lineages depending on the different conditions and sources in vitro.[26,32] Although MSCs offer a promising possibility for neural regeneration, their populations are heterogeneous and tend to exhibit distinct behaviors. It is a non-trivial task to enhance the survival of MSCs and to investigate the differential mechanism of MSCs in detail.[32] MSCs are sensitive to mechanotransduction; for example, they tend to differentiate into neural lineages and express neural markers on soft substrates.[32] The origin of MSCs plays a role in their plasticity, immunogenicity, and stemness.[33,34] For instance, bone marrow-derived cells can differentiate into astrocytes and neurons.[34] Li et al. composed a comprehensive review of MSCs for CNS repair and neurodegenerative disease.[34]

Unipotent stem cells, which include NSCs, have a narrow differentiation capacity and play a critical role in maintaining tissue organization.[26,35] NSCs have the potential to differentiate into neurons, astrocytes, and oligodendrocytes. Bond et al. and Uz et al. clearly elucidated that the fate of NSCs is influenced by intracellular regulatory mechanisms[36] and their biophysical environment, such as cellular co-culture and interactions between cells and ECM components.[35,37] Despite new techniques and insights emerging, there are still some hurdles to be overcome before NSCs can be implemented in human neurodegeneration. For instance, the heterogeneity of NSCs makes it difficult to differentiate between cells at different temporal states or identify distinct populations.[35] Problems that may arise during handling include cell survival rate and precise delivery of NSCs.[38]

2.1.3. Primary Cells

Primary cells are directly harvested from tissues or organs. They are usually a heterogenous cell population, exhibit a slower growth rate, are often post-mitotic, and have a comparatively shorter lifespan when kept in culture. Despite these disadvantages, primary cells are still one of the best models prior to conducting in vivo studies.[18]

Cortical neurons, illustrated in Figure 1b, are usually harvested from embryonic rodents. Typically, after a few days or a few weeks, neurons can be identified with the presence of axons, dendrites, dendritic spines, and synapses. Cortical neurons can form complex structures in vitro, e.g. synapses and neuronal networks, providing possibilities to research neurotransmission, plasticity, and network activity.[39,40] However, they also have some disadvantages, such as the need to sacrifice animal embryos.

Hippocampal neurons, shown in Figure 1b, are harvested from postnatal (P, in days) P 0–1 mouse pups, while cerebellar neurons are harvested from P 0–7 pups. Both sources of neurons are commonly used in primary neuron culture. Several hours after plating, both hippocampal and cerebellar neurons are able to develop axons, neurites, and even cell-to-cell interactions, making them suitable for live cell imaging and out-growth studies, e.g. growth cone studies.[41]

2.2. Ex Vivo Explant Culture

DRG explant culture from rodents is a widely-used model to study peripheral nerve regeneration. DRG neurons, depicted in Figure 1c, can be cultured from a wide range of postnatal stages, from embryonic stages (e.g., E13.5-E18 in mice) to adult animals, depending on the research requirements. The effect of axon outgrowth upon experimental treatment can be assessed by several criteria including axonal area.[42] Further axon injury can be applied with a scalpel or syringe needle, if necessary, to evaluate regeneration outcomes.

There are some common biological readouts for in vitro models and ex vivo explants. Pharmacological research and immunoblot are widely used to evaluate the effect of treatment on neural cells, as shown in Figure 1d,e. Visualization of neurite outgrowth analysis (e.g., for DRGs) and growth cones by immunostaining facilitate the evaluation of treatment efficacy. For example, comparing the diameter of neurite area allows us to compare the effect of axon outgrowth among different treatments, as illustrated in Figure 1f,g.

2.3. In Vivo Models

Analysis of in vitro models is an important first step to demonstrate the potential of biomaterials to stimulate neuronal growth. The next step is to provide in vivo evidence in small animal models, taking into account the complex interactions of many cell types at the injury site. For this, several in vivo PNS and CNS injury models in rodents are available, with some of them being introduced in this section.

The first in vivo model system to study nerve regeneration is the facial nerve injury model in rodents. The procedure of creating a facial nerve injury model is briefly described as follows. The facial nerve is transected (or crushed) at the trunk, buccal, or marginal branch depending on the research objectives. On the injured side, the function of the facial nerve is immediately disabled after injury, while the uninjured side serves as an internal control, as depicted in **Figure 2a**.[43]

A sciatic nerve mouse injury model is performed by crushing the nerve with a surgical clamp while the uninjured site serves as internal control, as shown in Figure 2b.[44]

SCI models can be induced by contusion, compression, distraction, transection, etc.[45] A hemi- or complete SCI is

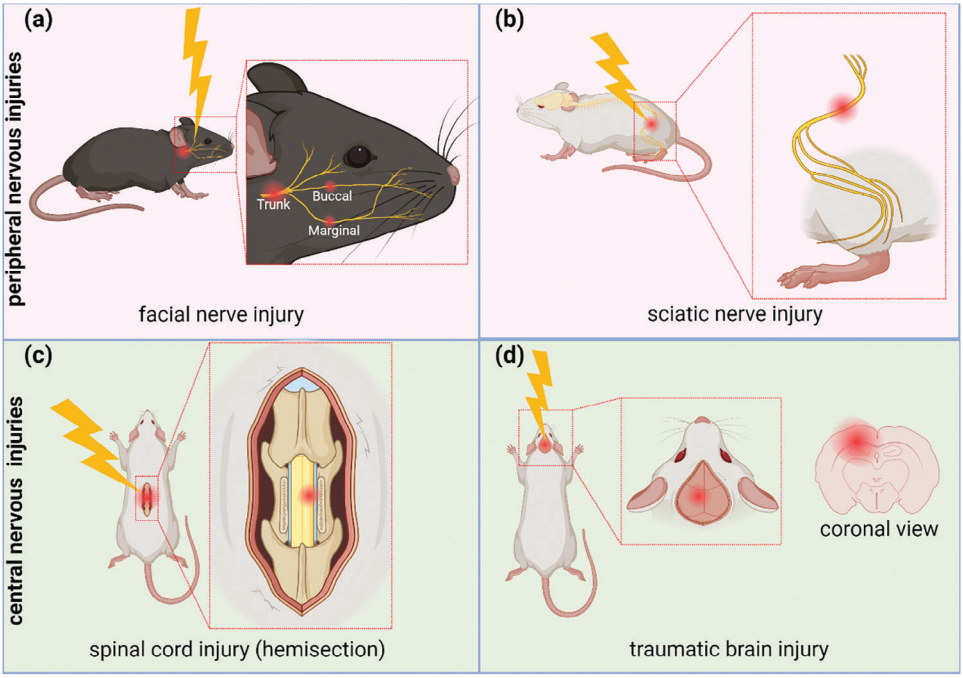

Figure 2. Examples of in vivo mouse models for PNI and SCI (generated using BioRender). a) facial nerve injury model. b) sciatic nerve crush model. c) SCI model. d) TBI model.

conducted by sectioning the spinal cord to induce injuries. The sensory and motor functions below the injury level are disabled depending on the severity of the injury, as illustrated in Figure 2c.[46]

There are different types of TBI models, such as the weight drop model, fluid percussion model, controlled cortical impact model, etc. A schematic of the weight drop model is depicted in Figure 2d.[47,48]

To analyze the recovery after injury in in vivo models, tests are performed to measure the behavioral differences between pre-injury and post-injury. For facial nerve injury, a high-speed camera is set up to record the whisker movement and evaluate the recovery after PNI, as depicted in **Figure 3a**. The footprint /Catwalk XT analysis is widely used in peripheral nerve injury models, particularly for sciatic nerve injury, as shown in Figure 3b.[49] Several behavior tests are suitable for central nervous system injury. To name a few, rotarod, open field, and novel object recognition tests exist and are illustrated in Figure 3c–e. In brief, the rotarod test records the time mice can stay on a rotating rod, and it is commonly used to analyze the motor function of mice.[49] In another type of test, a mouse is allowed to run an open-field test to analyze the locomotor function.[50] The novel object recognition test analyzes the memory function in which a healthy mouse is supposed to spend more time with the novel object.[51]

3. Peptide-Based Approaches Toward Neuronal Regeneration

Natural biomaterials, such as collagen, gelatin, hyaluronic acid, alginate, and chitosan, have been utilized for neural regeneration.[52] They hold the advantages of inherent bioactivity and biodegradability because they stem from acellularized tissue and extracellular matrix-derived macromolecules. Large-scale synthesis is another benefit of using natural biomaterials for neuronal regeneration. However, batch-to-batch variability in production and an uncontrollable degradation profile are drawbacks that need to be considered.[1,2]

In contrast to natural biopolymers, synthetic polymers, like polycaprolactone, poly-L-lactic acid, and polyethylene glycol (PEG), are another category of biomaterials implemented for neural regeneration.[8] They are known for their high reproducibility, processability, and tunability, which compensate for the shortcomings of natural biomaterials. However, they lack biological cues due to their chemical nature; thus, more synthetic approaches to decorating bioactive motifs on synthetic polymers are necessary to increase their biocompatibility. It is also worth noting that the degradation product of lactic acid-based materials acidifies the local physiological environment, which may be disadvantageous.[1,2]

Synthetic peptides, consisting of amino acids, offer an alternative class of biomaterials for neural repair, with non-cytotoxic degradation products. Based on sequential designs, they can incorporate bioactive groups and synchronously serve as a matrix for cell adhesion. Moreover, synthetic peptides can be synthesized with high purity using commercially available automated synthesizers.[2,53] Combining the advantages of both natural biomaterials and synthetic polymers, synthetic peptides provide new possibilities for neural tissue engineering. In the following section, we categorize therapeutic peptides into soluble peptides or extracellular matrix (ECM) mimics. Soluble peptides typically do not form assembled structures and circulate around the body,

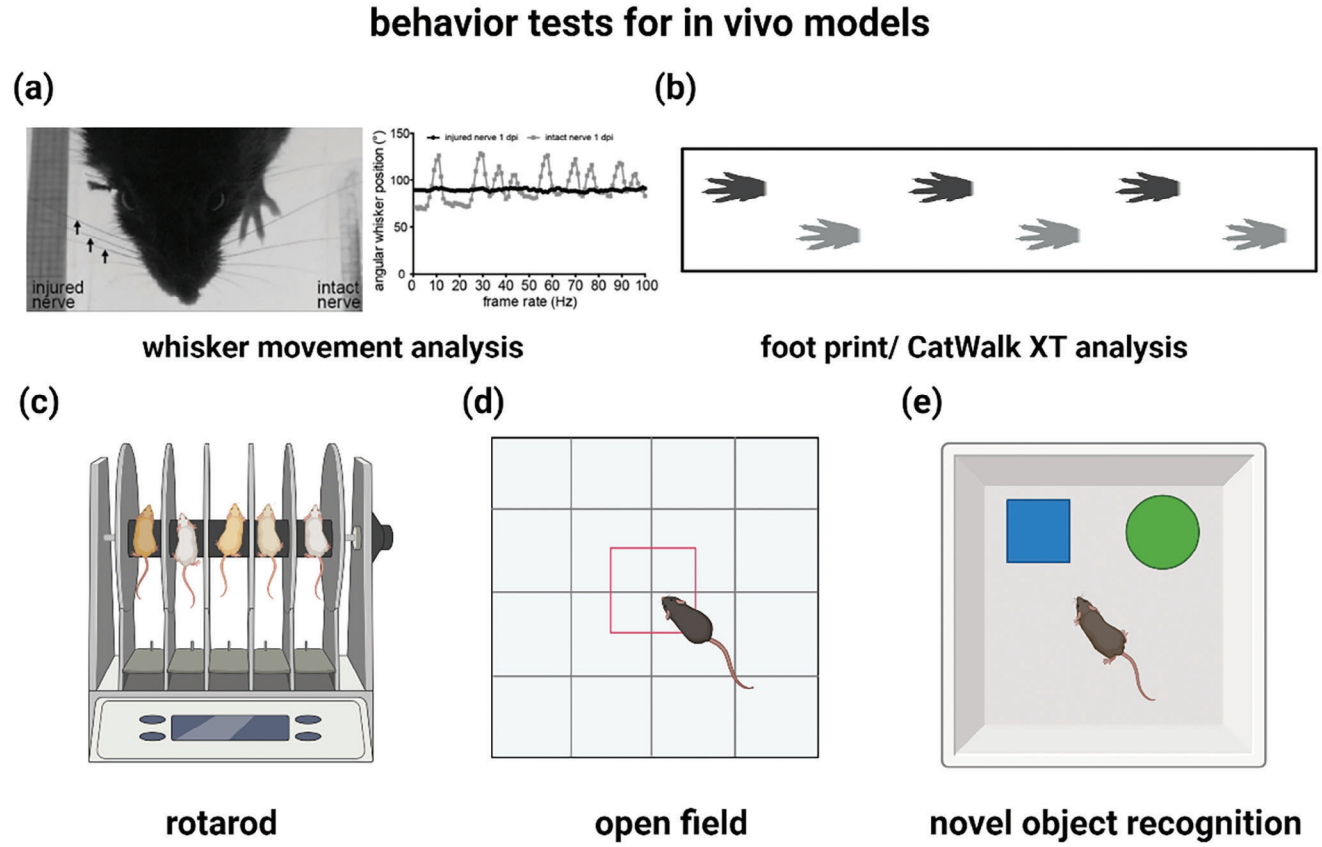

Figure 3. Behavior tests for in vivo models (generated using BioRender). a) a whisker movement analysis, video shows disability of whisker movement on the injury side (left) and the registered position of whiskers on both sides (right). Reproduced with permission.[43] Copyright 2019, WILEY-VCH GmbH. b) Footprint/ CatWalk XT analysis records several gait parameters. c–e) The schematics of rotarod test, open field test, and novel object recognition test.

while self-assembling peptides (SAPs) remain solid forms and act as ECM mimics.

3.1. Soluble Peptide

As the previous sections elucidated, the BBB or the BSCB are the barriers that maintain homeostasis and control the permeability of any substance to the CNS. However, these barriers also prevent small molecule drugs from reaching an injured nerve tissue. So-called cell-penetrating peptides (CCPs) can facilitate the transport of drug molecules across biological barriers like the BBB and BSCB, as illustrated in **Figure 4**. The transactivator of transcription (TAT) protein of HIV-1, one of the most well-known CPP, was discovered in the 1980s and was soon utilized to transport molecules intracellularly. CPPs are a group of peptides, consisting of 5 to 30 amino acids, which go through cell membranes by energy-dependent or -independent mechanisms.[54] In addition, they can be further classified based on physicochemical properties, including cationic, amphipathic, and hydrophobic CPPs or their origins. The cellular uptake mechanisms of CPPs are direct penetration, endocytosis, or translocation by a transitory structure such as a micelle.[54,55] CPPs alone have high transportation efficiency across cell membranes, but the overall efficiency can be affected by the molecules (e.g., size and charge) coupled to CPPs.[55] There are various types of CPPs designed to act as imaging agents when coupled to dyes or for the delivery of peptides, proteins, and small drug molecules.[54] There are also specific examples of CPPs applied to SCI repair, where the TAT peptide was coupled with phosphatase and tensin homolog (PTEN) antagonist peptides. The PTEN-TAT peptide conjugate promoted axonal repair and functional recovery after SCI in adult mice.[56,57]

Next to peptides that enable the crossing of cellular barriers, targeting peptides can also be useful for delivering molecules of interest to specific regions in the body. Mann and colleagues reported a short peptide sequence, CAQK, that selectively binds to structures in the injured mouse and human brain.[58] The authors performed proteomics analysis of proteins separated from injured brain extracts by affinity chromatography on immobilized peptides. The CAQK sequence bound predominantly to the lectican family of CSPGs, in particular versican and Hapln4. This sequence was then tested as an imaging agent by labeling it with a commercially available fluorophore or a potential drug carrier via conjugation with silicon nanoparticles encapsulating oligonucleotides. In a mouse TBI model of controlled cortical impact injury, no material was detected in the liver or skin of the experimental group, suggesting that the CAQK peptide has low toxicity and high binding affinity to only the injury site in the brain. The ex vivo histological study of the brain confirmed that the material was mainly attached to the cortex and the corpus callosum

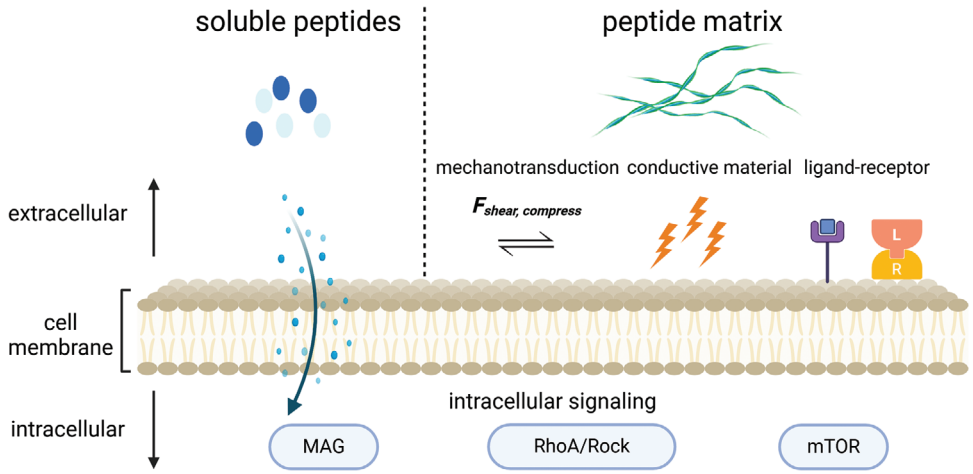

Figure 4. A schematic of extracellular and intracellular stimuli provided by peptide-based materials for neural regeneration (generated using Biorender). The left side shows that soluble peptides have to go through cell membranes or barriers to active intracellular cascades for neural regeneration. The right side illustrates how SAPs as ECM mimics can trigger intracellular cascades, such as MAG,[10] Rho-associated protein kinase (RhoA/Rock),[3,10] mammalian target of rapamycin (mTOR),[8,10] etc., for neural regeneration, through ligand-receptor pairing, mechanotransduction, and electrical stimuli.

areas, while the material was nearly undetectable in the uninjured group. This phenomenon was also confirmed in the in vivo mouse model.[58] The CAQK peptide can be an appealing candidate to be coupled with neurotrophic molecules or PTEN antagonist peptides and carry these therapeutic molecules to the TBI site.

Soluble peptides (CPP or targeting peptides) as drug delivery systems have improved over the last few decades, with soluble peptide conjugates exhibiting the ability to transport various molecules across cellular or tissue barriers.[55] Only very little research has been done on soluble peptides for neural regeneration so far and to expand the use of them for neural regeneration, some obstacles need to be overcome (e.g., low cell specificity, cytotoxicity at high dosage, and short half-life in vivo). Additionally, cellular uptake mechanisms need to be elucidated, as the internalization of soluble peptides is limited to some cell types. Some recent examples demonstrate that targeted drug delivery can be improved when CPPs are coupled through responsive groups (e.g., redox, pH, etc.) that enable a triggered release of the drug in the target tissue.[54] In a recent review, Chagri and coauthors highlighted physiological stimuli, such as pH, glutathione, reactive oxygen species, enzymes, etc., which allowed the soluble peptides to target specific cellular environments.[59] Last but not least, the metabolic stability of soluble peptides has been improved in vivo by substituting L-form amino acids with non-natural amino acids or D-form amino acids, which are less sensitive to enzymatic degradation.[54] Although soluble peptides are not yet a popular choice of therapeutics for neural regeneration, soluble peptides that overcome the previously mentioned hurdles are still feasible candidates for neural repair.

3.2. Extracellular Matrix Mimics

The ECM is an essential component of all tissues, providing mechanical support and barrier function for the structural integrity of multicellular organisms. Furthermore, the ECM functions as a reservoir for signaling cues thereby regulating cell fate. In the design of functional biomaterials for neural regeneration, scientists are aiming to emulate those cues provided by the ECM.[60] In neural tissue, the ECM is a fibrous network, primarily consisting of proteins, proteoglycans, and glycosaminoglycans. The ECM environment surrounding neurons is complex because of its multiscale architecture, various cell types, multiple biomolecules, fibrous networks, and interpenetrating vascular systems.[61] Therefore, mimicking the neural ECM necessitates incorporating as many aspects of the neural environment as possible.

SAPs are a class of materials consisting of short peptide sequences and show potential for mimicking the neural ECM. They form nanofibers thereby reflecting essential properties of the neural ECM under physiological conditions.[62] SAPs form their structure via noncovalent interactions, for instance, hydrogen bonding, π–π stacking, electrostatic interactions, and van der Waals interactions. Although non-covalent interactions are generally weaker than covalent bonds, these weaker but directional interactions give rise to reversibility, stimulus responsiveness (e.g., redox, pH, etc.), and nanostructures of SAPs, which resemble tubulin proteins inside of cells.[63] SAPs are solid or gel matrices that can potentially assemble and attach at the site of interest (e.g., injection at the injury site), which not only allows minimally invasive surgery but also bypasses the BBB or the BSCB.[53] Additionally, SAPs are easily modified with aromatic groups, lipid chains, and biomolecules to tune the physical (i.e., topography) and biochemical (i.e., growth factor mimic) properties of the neural ECM.[62,63] Furthermore, with appropriate sequence design and control over the assembly pathway, hierarchically ordered, and aligned structures can be produced which in turn influence cell behavior.[64,65]

With these attractive features of SAPs, this class of materials is a promising ECM mimic for neural regeneration. In the following sections, we expand on how the physical and biochemical properties of the ECM influence neural behavior and update and highlight some examples of SAPs for neural regeneration.

4. Impact of Matrix Properties on Neural Behavior

Mimicking the ECM requires more than merely providing a biocompatible niche for neural cells to attach to. The conceptual schematic shown in Figure 4 illustrates how the physical and biochemical properties of the ECM strongly influence neural behaviors, such as cell adhesion, cell migration, cell morphology, cell signaling pathways, cell differentiation, and neurite outgrowth.[66]

4.1. Physical Properties

The following sections will explain how physical properties affect neural cells and provide some strategies and examples for tuning the physical properties of SAPs to enhance neural regeneration.

4.1.1. Matrix Stiffness

Mechanotransduction refers to the process of cells sensing and modifying their behavior in response to the ECM stiffness and mechanical forces, such as osmotic pressure, shear force, and compressive load from the surroundings. This process regulates tissue-specific differentiation and maintains tissue homeostasis.[67] For example, the interruption of interstitial fluid in the brain disrupts the balance between Ca^{2+} and Na^{1+} ions, thus, mechanically stimulating axons and triggering Ca^{2+}-mediated neurotoxicity.[68] In terms of designing a matrix for neural repair, the stiffness of neural tissue depends on the cell type and location. Tissues in the PNS are generally stiffer than those in the CNS because of more aligned nerve fibers and protective connective tissue in the PNS.[2] In contrast, the brain ECM consists mostly of interstitial fluid, which gives rise to viscoelastic properties and a low stiffness of brain tissue.[2,69] More comprehensive mechanisms of how mechanotransduction influences axon outgrowth are systematically reviewed elsewhere.[70]

At the cellular level, Koser et al. reported that axons of retinal ganglion cells grow toward softer tissue, mediated by mechanosensitive ion channels.[71] NSCs form synapses and are more prone to differentiate into neurons with neurite extension and branching on a soft matrix (0.1–0.5 kPa). In contrast, a stiffer matrix (0.5–10 kPa) stimulates NSC migration and differentiation into glia cells.[2] MSCs and pluripotent embryonic stem cells tend to express higher levels of neural markers when cultured on a soft matrix (0.1–1 kPa).[67] It was reported that a stiffer matrix (above 200 KPa or scar tissue) reduced the viability of neural cells in both an in vitro study and in the clinic.[2] Typically, SAPs form a rather soft matrix, which is beneficial for mimicking the mechanical properties of natural brain tissue and it may be possible to create materials, possibly in combination with covalent polymers, with anisotropic mechanical properties that better reflect the complexity needed for improving neural regeneration in the future.

4.1.2. Topography

Neurons have a polarized morphology with soma and dendrites for receiving biological signals from other neurons, and axons for sending biological signals outward. Random neurite growth results in an inefficient neural network.[66] Udvary et al. published that neuronal morphology also has an impact on cellular behavior and the architecture of neural networks.[72] This phenomenon indicates that the architecture of the neural environment and neuronal morphology complement each other. The environment of neural cells is multiscale and influences cell behavior, such as adhesion, alignment, and morphology.[2,69] Vedaraman and colleagues implemented a two-photon lithography technique to fabricate precise substrates, suggesting that substrates with anisotropic (having different physical properties in different directions), discontinuous geometry at the micrometer scale can support the alignment of dorsal root ganglia.[73] From a microscopic scope, Yang and coauthors published a review on the correlation between neural regeneration and different topographies. For continuous topography, embryonic hippocampal neurons tend to grow in parallel in deeper grooves and grow into multipolar cells when the grooves are wider. For discontinuous topography, PC12 cells grew fewer and shorter neurites on smooth surfaces, as illustrated in **Figure 5**. For random topography, hippocampal neurons showed higher survival rates on surfaces with nanoroughness.[66] These findings suggested that different topographic features on the same type of substrate influence the behaviors of neural cells.

In addition to fabricating anisotropic substrates, a peptide nanofiber hydrogel composed of aligned fibrin and functionalized SAPs was prepared for functional recovery after PNI.[74] The aligned fibrin structure was first electrospun into a construct and soaked in the solution of SAP and growth factor mimic-functionalized SAP to obtain an interpenetrating peptide nanofiber hydrogel. Under scanning electron microscopy, the pure aligned fibrin showed uniform and smooth microfibers. In contrast, the hybrid exhibited aligned microfibers surrounded by dense nanofibers. Hybridization of this construct with functional SAP increased surface roughness for improved cell adhesion, enabled the adjustment of substrate stiffness, and provided an aligned structure for directional SC outgrowth.[74] These findings exemplified that SAPs introduced structural heterogeneity, which is an important aspect of the neural ECM.[69] Tran et al. encapsulated carbonyl iron microparticles in a SAP monomer solution, enabling magnetically-induced alignment of peptide nanofiber hydrogel. Compared with the non-aligned nanofibers, the aligned structure stimulated MSCs to express higher levels of brain-derived neurotrophic factor (BDNF) and exhibit anti-inflammatory effects, while neuron progenitor cells increased the number of infiltrating axons. This work provided a new strategy to tailor the mechanical properties and topography of the matrix for SCI repair.[75] These examples show that controlling topographical cues is very challenging with pure peptide systems. However, when combined with (bio-)polymers, peptides can lead to significantly improved outcomes in nerve growth.

4.1.3. Epitope Presentation on SAPs

The spatial arrangement of epitopes or growth factor-mimicking peptides is critically important for efficient cell adhesion and correct molecular recognition. Compared with the mesoscale topographical effect, this section highlights how molecular design at nano-scale can influence cellular behavior. Epitopes

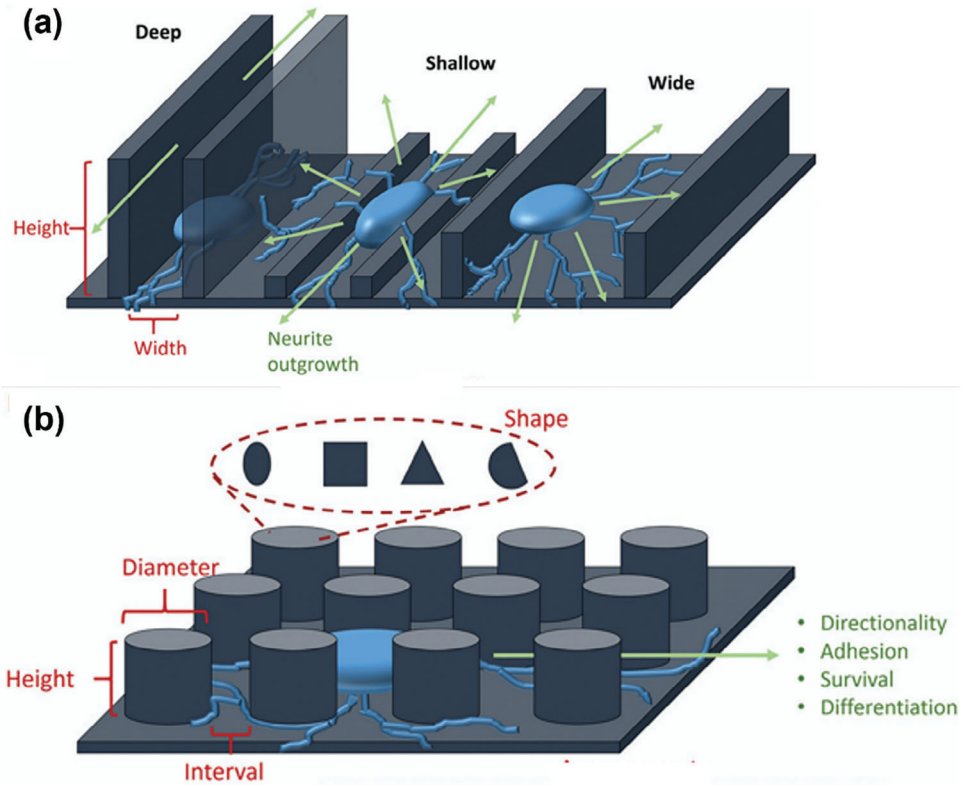

Figure 5. A schematic of topographical effects on neural cells. a) Neural cells in blue experience continuous topographies, provided by defined grooves. The depth and width of the grooves can affect the direction of neurite outgrowth. Adapted with permission.[66] Copyright 2021, Royal Society of Chemistry. b) Neural cells in blue encounter discontinuous topographies established by randomly positioned pillars. These pillars with different diameters, heights, intervals, and shapes can influence the adhesion, survival, and differentiation of PC12 neural cells as well as the alignment of neurite outgrowth. Adapted with permission.[66] Copyright 2021, Royal Society of Chemistry.

that are presented on SAPs enable easy adjustment of various factors that determine cell receptor binding, such as overall epitope concentration, epitope spacing, etc.

Controlling sub-nanometer epitope spacing by an SAP hydrogel is one strategy to statically tune the spatial arrangement between matrix and cells, as illustrated in **Figure 6**a. In this work, Pashuck et al. designed 2 fibronectin epitopes and site-specifically conjugated the epitopes on a linear SAP with 3 different sub-nanometer distances ranging from 0.7 nm to over 6 nm. Human umbilical vein endothelial cells (hUVECs) showed the most promising bioactivity for SAPs presenting 2 epitopes with 3.2 nm apart. This study demonstrated that the

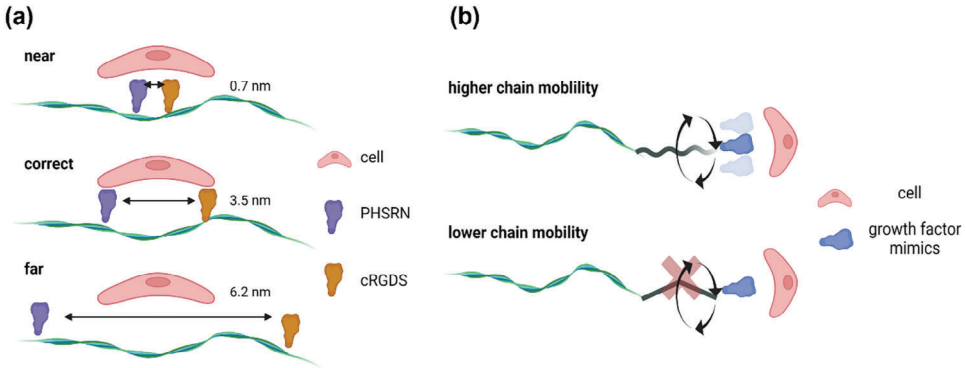

Figure 6. A cartoon illustration of the spatial arrangement of epitopes presented statically and dynamically in SAP systems (generated using BioRender). a) Subnanometer epitope spacing was achieved by site-specifically conjugation of epitopes on a linear SAP. The correct or suitable distance of epitopes on the SAP backbone increases the specificity in targeting cell surface proteins.[76] b) Molecular chain dynamics of epitopes presenting PA were adjusted based on chain motion such as vibration and rotation. Higher molecular chain dynamic activity increases the efficiency of epitopes being presented to the receptor on the cell membrane, leading to improved neural regeneration.[77]

proper design of SAPs allowed for the tuning of the location and density of epitopes presented to cells at the nano-scale level, thereby precisely modulating the cellular response.[76] This research pioneered investigations on how sub-nanometer epitope spacing could influence cellular responses. We believe that this concept also applies to neural regeneration as epitope spacing can be designed to match surface protein localization on neural cells.

The molecular design of SAPs is not only limited to the distance or density of the epitope but also influences molecular chain dynamics to optimize the epitope presenting efficiency for neural cells, depicted in Figure 6b. Peptide amphiphiles (PAs) are a class of materials containing a functional group and a structural forming peptide sequence linked to a hydrophobic alkyl tail. Álvarez and coauthors found that the mobility of the molecular chains could be adjusted by mutating the position of the tetrapeptide sequence (i.e., Ala, Glu, Gly, and Val) connecting the 3 domains of the PA. The design and hypothesis were confirmed by molecular dynamics simulations. After tuning, the epitope and growth factor mimetic peptide-functionalized PA became more mobile, leading to higher expression of neural markers and therapeutic effects in an in vivo SCI model.[63,77] In the future, greater efforts should be directed at understanding the role of dynamics and mobility in regard to epitope presentation on ECM mimics.

4.1.4. Co-Assembling

Co-assembling is the combination of 2 or more distinct monomers in supramolecular assemblies via complementary intermolecular interactions, for instance, charge-charge interaction, π-π interactions, and dipolar moment.[78] Controlling the properties of various peptide monomers is challenging as they can undergo disruptive, cooperative, or orthogonal assembly, i.e., depending on the types of peptides that are mixed together their assembly can be prevented, enhanced, or remain unaffected, respectively.[79] Nonetheless, this approach is still appealing for biomedical applications as the co-assembly of peptides can tune the mechanical properties and integrate multiple biological motifs into one homogenous architecture for a synergistic effect on the cellular response via the spatial and chemical arrangement of multiple bioactive signals.[78,79] Understanding the molecular arrangement and mechanism of co-assembling or self-sorting peptides from empirical data, computer simulation, and real-time imaging could improve the design of new co-assembling peptides for different applications.[80–82]

Gelain's group co-assembled 2 complementary charged SAPs to form a composite SAP hydrogel. The authors applied computer simulations to study the co-assembled hydrogel and propose the co-assembly mechanism and potential structural arrangements. Imaging revealed that the co-assembled hydrogel contained a longer and more abundant fiber structure compared to individual peptide assemblies. The rheological data suggested that the co-assembled hydrogel had higher stiffness than the 2 counterparts did, which was consistent with the matrix stiffness for neuron culture. Additionally, both human and murine NSCs survived and differentiated well on the co-assembled hydrogel. Employing an SCI model also displayed the neuroregenerative potential of the co-assembled hydrogel.[80] On top of adjusting the molecular packing and tuning the stiffness of the SAP matrix, introducing multiple epitopes or growth factor mimics with synergistic effects is another advantage of implementing a co-assembly strategy. There are several groups using co-assembling methods with different SAP backbones, namely, the fibronectin and laminin-derived sequence modified with fluorenylmethoxycarbonyl (Fmoc), a protecting group for site-specific chemical reaction. Those nanofibers have been used for modulating muscle progenitor cells,[83] NGF mimic and BDNF mimic functionalized SAP for facilitating peripheral nerve regeneration, and heparan sulfate mimic and laminin-derived sequence decorated PAs for SCI repair[85] to name a few.

4.1.5. Conductive Biomaterials

Electrical signals influence cell behavior through the membrane potential, ion channel activation, and electrophysiological state, resulting in proliferation, migration, and differentiation.[2] These electrical cues are crucial for neural cells as they are responsible for signal propagation.[2] Therapeutic approaches use implantable conductive materials that enable propagation of electrical signals, e.g. between neuronal cells, but also external electrical stimulation has been shown to be effective, illustrated in **Figure 7**.[84,85] Hybrid conductive materials are used for neural regeneration, in which conductivity is derived from conductive polymers, carbon-based materials, or metals. Most studies of conductive biomaterials for neural regeneration are limited to in vivo studies Common drawbacks of applying conductive materials in further in vivo studies or clinical trials for neural repair are limited because of their solubility, biocompatibility, long-term stability, and consistent conductivity.[84,85]

In one example, a SAP-carbon nanotube hybrid hydrogel was designed to contain amino acids with aromatic groups and with imidazolium functionalization at the N-terminus of the SAP to increase the intermolecular cation$-\pi$ interaction and $\pi-\pi$ stacking. The introduction of an imidazolium group in the SAP system helped solubilize and stabilize the carbon nanotubes in the hybrid hydrogel. The in vitro study suggested that treating DRG neurons with the hybrid material and an external electrical stimulus promoted axon extension and myelination.[87] In another study, Arioz et al. identified a tetra(aniline)-conjugated peptide nanofiber and tested its potential for PNI repair. Unlike other PAs, this PA was designed with hydrophilic, β-sheet forming moieties, and a conductive oligomer as the hydrophobic tail. The conductivity of the hybrid peptide hydrogel was not measurable due to the insulating peptide backbone but was sufficient to facilitate the differentiation of PC12 cells.[88]

However, it is worth noting that even without using conductive materials implanted into a patient, electrical stimulation has demonstrated significant advantages in promoting nerve regeneration. For example, Courtine's lab successfully conducted one of the few clinical cases using non-invasive spinal cord electrical stimulation (ARC^{EX} Therapy) for treating SCI.[86] Therefore, we envision that the combination of implantable conductive biomaterials and non-invasive electrical stimulation for synergistic

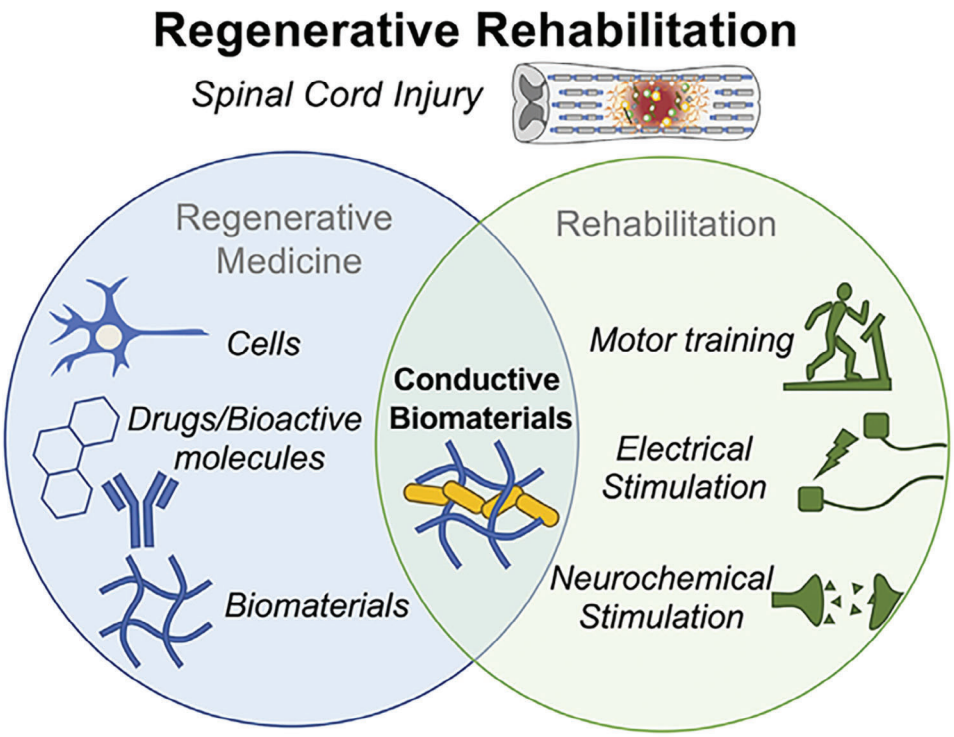

Figure 7. A Venn diagram of conductive biomaterials for regeneration and rehabilitation of SCI. At the cellular level, conductive biomaterials can stimulate cells and facilitate the release of drugs and bioactive molecules. For rehabilitation, conductive biomaterials provide neurochemical stimulation electrical stimulation, and motor training. Adapted with permission.[85] Copyright 2022, Elsevier.

therapeutic effects can improve the well-being of patients suffering from PNI and SCI.

4.2. Biochemical Properties

Cells communicate not only via physical cues but also through the structural and signaling proteins present in the ECM. Interestingly, it was found that full proteins are not always required to achieve a desired biological response (attachment, survival, differentiation, etc.) from the cell. Small molecules or peptides that mimic the essential regions of the full protein can be sufficient to achieve comparable outcomes. When coupled to a matrix, such mimics tend to be more stable and less sensitive to the environment than their full protein counterparts. Peptide epitopes on SAP nanofibers can actively interact with cells and initiate specific cell signaling pathways, in some cases with greater efficiency than endogenous proteins.[89] In the following section, functional peptide sequences known to affect neural functions such as neural regeneration are categorized into cell adhesion epitopes and growth factor mimics. Examples of sequences functionalized on SAPs are covered in the following sections and summarized in **Table 1**.

4.2.1. Cell Adhesion Epitopes

Cell adhesion plays a critical role in facilitating cell-cell and cell-matrix interactions, enabling the transmission of environmental information to activate intracellular signaling.[89] Adhesion proteins, such as vitronectin, fibrinogen, laminin, and fibronectin, contain multiple integrin ligands that interact with cell receptors such as integrin receptors responsible for cell adhesion.[89,90]

There are many peptide epitopes derived from fibronectin, such as RGD, PHSRN, and GRGDSP. Among them, the RGD peptide is the shortest and most investigated sequence, along with its analogs for cell adhesion.[91] Laminin-derived peptide epitopes, including IKVAV, YIGSR, and other derivates, have been used in SAP and PA systems to explore their potential for neural repair.[89,92] An octapeptide derived from tenascin-C, a class of glycoproteins, is involved in the regenerative progress of many tissues.[89] Sever et al. published tenascin-C decorated PA nanofibers, which enhanced the neurite outgrowth of PC12 cells.[93] The collagen superfamily consists of 46 different polypeptide groups, 28 of which can self-assemble into supramolecular structures. Collagen type I sequences, DGEA and GFOGER (O = hydroxyproline) have been conjugated to SAPs for bone tissue engineering.[89] Jain and colleagues developed nanofiber hydrogels based on a collagen-inspired peptide with the sequence FFGSO and co-assembled it with a laminin-derived peptide. These hydrogels showed high biocompatibility with both fibroblasts and glioma cells.[94] Cadherins, unlike the aforementioned cell adhesion peptides, are transmembrane cell receptors that facilitate cell-cell communication for cell migration and maintenance of tissue structure.[89] Roy's lab reported a SAP nanofiber system based on cadherin-derived

Table 1. Summary of different SAP backbones functionalized with growth factor mimics or epitopes and their respective in vitro and in vivo experiments.

Function	Protein source	Epitope	Backbone	Cell	In vivo models	Reference
Cell adhesion	Cadherin	HAVDI	Fmoc, Nap	C6	X	[93]
	Fibronectin	RGD	α-Helical-peptide	m-NSC	X	[108]
	Laminin	IKVAV, YIGSR	Fmoc	C6 SH-SY5Y	X	[90]
	Tenascin-c	VFDNFVLK	Lauric acid	PC12	X	[91]
Neurotrophic factor	BDNF	RGIDKRHWNSQ	RADA	r-SC r-DRG PC12	r-PNI sciatic nerve	[81]
				r-SC h-UVEC	r-PNI sciatic nerve	[101]
	CMX-9236	KKDGDGDFAIDAPE	MDP	r-primary cortical neuron	r-TBI fluid percussion	[111]
	NGF	CTDIKGKCTGACDGKQC	RADA	r-SC r-DRG PC12	r-PNI sciatic nerve	[81]
	Netrin-1	CIDPC	Palmitic acid	m-primary cortical neuron	X	[124]
Angiogenesis	FGF	YRSRKYSSWYVALKR	Palmitic acid	h-UVEC	X	[98]
	Osteopontin	SVVYGLR	RADA	r-NSC h-UVEC	z-TBI embryo toxicity test	[107]
		RPKPQQFFGLM	RADA	r-primary cortical neuron	r-SCI	[97]
	PDGF	VRKKP	Nap+FF	m-NSC	r-SCI	[100]
	VEGF	KLTWQELYQLKYKGI	RADA	r-SC h-UVEC	r-PNI sciatic nerve	[101]
			MDP	r-primary cortical neuron	r-TBI fluid percussion	[96]

h is human; m is a mouse; r is a rat, and z is zebra fish; X stands for no in vivo experiment conducted.

peptides with aromatic moieties to modify the N-terminus. The SAP hydrogels promoted the proliferation of fibroblasts and neurite extension.[95]

Cell adhesion, involving integrins and cadherins, is an essential mechanism for cell-ECM communication that is ubiquitous across different cell types. Growth factor activation, on the other hand, is relatively cell-type selective.

4.2.2. Growth Factor Mimics

Growth factors regulate essential cellular processes, including cell proliferation, migration, and differentiation. The mechanisms rely on cellular receptors sensing and binding to growth factor ligands to activate intracellular cascades that reach the nucleus and influence transcription. Unlike full-length proteins which are prone to rapid denaturation, coupling growth factor mimics to artificial matrices provides a stable alternative while preserving the reactivity and specificity of the original.[89]

The growth factor mimics of BDNF and NGF can promote neurite outgrowth and neuroprotection, like their full-length protein counterparts.[89] Lu et al. investigated the therapeutic effects of SAPs modified with BDNF-derived peptides in an in vivo SCI model. Their results, such as electrophysiological recovery and motor functional analysis, showed promising potential in the treatment of SCI.[96] Other growth factor mimics or neurotrophic peptides, which promote neurogenesis, are summarized elsewhere.[89,91,97]

The angiogenesis pathway garners significant interest from many research groups because the vascularization of tissue improves oxygen supply and metabolism, which is essential for tissue repair. A vascular endothelial growth factor (VEGF)-derived peptide coupled to SAPs not only increased blood vessel density in a rodent model but also improved cell survival.[98] Substance P, derived from osteopontin, is an angiogenic motif that improves adhesion, migration, and tube formation of endothelial cells. This motif was attached to SAPs and shown to be angiogenic and neurogenic in a rat SCI model.[99]

Fibroblast growth factor (FGF-2) regulates cell survival, differentiation, and homeostasis during tissue development. PAs modified with FGF-2-derived peptides facilitated the proliferation and migration of hUVECs in vitro and boosted cell survival and angiogenesis in a rat SCI model.[77,100]

Platelet-derived growth factor (PDGF) can promote angiogenesis and synaptogenesis, prevent neuronal death, and direct the differentiation of NSCs into oligodendrocytes and neurons.[101,102] PDGF mimetic peptides were coupled on 2 self-assembling motifs, naphthylacetic acid and diphenylalanine (FF), to trigger hydrogel formation for NSC encapsulation. This cell-laden SAP hydrogel attenuated the inflammatory response at the injury site, enhanced angiogenesis, promoted remyelination, and remarkably improved functional recovery in a rat SCI model.[102]

Co-assembly of different functional SAPs for synergistic biochemical effects is also one strategy to maximize the therapeutic

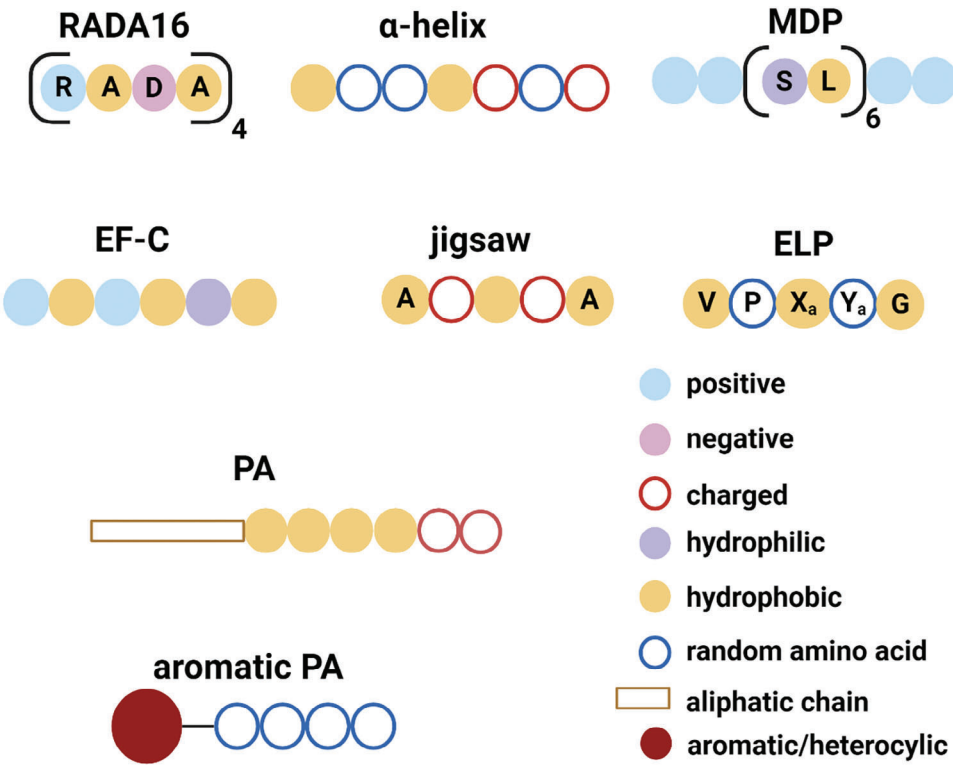

Figure 8. A schematic of different amino acid building blocks of SAPs. Different icons represent different amino acids, where Xa stands for either glycine or alanine and Ya can be any amino acid other than proline (generated using Biorender).

potential of SAPs. Lu et al. investigated the synergistic effect arising from the co-assembly of SAPs decorated with NGF and BDNF mimetics on PC12, SCs, and DRG cells in vitro. Furthermore, the co-assembled SAPs were found to bolster axonal regeneration and functional recovery in a murine PNI model.[83] Another study on PNI repair investigated the co-assembly of VEGF and BDNF coupled to SAPs. The co-assembled SAP stimulated the myelination of SCs and adhesion of hUVECs and promoted angiogenesis and neurogenesis in vivo.[103] Co-assembly of laminin-derived peptides (i.e., IKVAV) and FGF-2 modified on PAs can synergistically activate 2 biological pathways. Alvarez et al. found that these co-assembled PAs induced remyelination and increased vascular area fraction, length, and branching. In a SCI model, these PAs also induced significant angiogenesis and functional recovery.[77]

Besides these established peptides, new functional peptide sequences can be revealed by applying phage display, computational screening, and artificial intelligence.[89] The coupling of various peptide epitopes or growth factor mimics onto SAPs is straightforward. However, it is challenging to increase the accessibility of functional domains on SAPs for several reasons: Poorly soluble peptide epitopes have limited exposure to the aqueous solvent and may be buried in the self-assembled structure. A spacer of the correct length may be necessary to facilitate efficient receptor binding. Furthermore, high concentrations of epitopes may interfere with the self-assembly behavior of SAPs and result in altered structures.[89,91]

We anticipate that significant advances will be made in the near future in creating ECM mimics with tailored bioactivity through the optimized presentation of bioactive epitopes. This is due to a better understanding of the complex interplay between different biological signals necessary for nerve regeneration supported by more powerful computational methods.

5. Generation of Self-Assembling Peptide Matrix

There are various supramolecular self-assembling structures based on different designs of the amino acid sequence, including α-helix, β-sheet, collagen-like, elastin-like, multidomain, PA, aromatic peptide amphiphile, etc. The amino acid building blocks of SAPs are illustrated in **Figure 8**. In the following sections, we elaborate on different peptide backbones and provide examples of SAPs for neural regeneration, summarized in Table 1.

5.1. RADA

Derived from the yeast protein, RADA16 (RADARADARA DARADA), EAK16 (AEAEAKAKAEAEAKAK), and their SAP variants are ionic self-complementary peptides, shown in Figure 8. The design principles of this class of peptides are 1) alternating hydrophilicity of amino acids, 2) alternating positively and negatively charged amino acids, 3) alternating chirality of natural L- or unnatural D-amino acids, and 4) alternating polarity of amino acids.[104] The chirality, charge arrangement, and length of the amino acid sequence play important roles in defining the nanostructure and mechanical properties of these SAPs.[104,105] For example, LDLK12 (LKLKLKLKLKLK) and LDLD12 (LDLDLDLDLDLD) are SAP sequences designed and

modified based on RADA16 and EAK16 as neural scaffolds with very similar properties, due to the strictly alternating hydrophobic and charged pattern of amino acids.[53,106,107]

RADA16 was functionalized with BDNF and applied to astrocytes and human umbilical cord MSCs. The functionalized RADA16 SAP hydrogel provided mechanical support in the lesion site to ensure cell survival and BDNF to stimulate cell adhesion.[108]

In a different strategy, an angiogenic motif SVVYGLR derived from osteopontin, was modified on the RADA16 SAP hydrogel to promote the adhesion, migration, and tube formation of hUVECs. An in vivo optic tectum wound model in the brain of adult zebrafish was conducted to prove the neurogenesis of this SAP hydrogel.[109]

Lu et al. conducted research in the co-assembly of multiple growth factor mimetics modified with RADA SAPs. In one case, the co-assembly of BDNF-SAP and VEGF-SAP for PNI repair was investigated, illustrated in **Figure 9**a–d. This co-assembled SAP hydrogel synchronously induced neural regeneration and angiogenesis in an in vitro study. When the functionalized SAP was filled into a chitosan nerve conduit, the therapeutic effect was comparable to the autograft for sciatic nerve repair.[103]

5.2. α-Helical Peptides

α-helical peptides are mainly composed of a repeating unit of 7 amino acids, abcdefg, with hydrophobic groups (a and d position) and charged groups (e and g position) to mediate assembly, as illustrated in Figure 8. The other residues (b, c, and f) are exposed on the surface of the assemblies and differ in design. The self-assembled configuration can be tuned by controlling conditions, such as temperature, pH, and ionic strength.[53,105] Woolfson's lab designed self-assembling fiber hydrogels based on α-helical peptides to facilitate the attachment and migration of murine embryonic NSCs. The SAP was first modified with an azido group and allowed for post-modification with alkyne-decorated RGDS peptide to enhance cell adhesion. NSCs expressed more neural lineage markers (microtubule-associated protein-2, MAP2) and fewer glial lineage markers (GFAP) when cultured on the RGDS-modified SAP. This indicated that α-helical peptide SAPs modified with peptide epitopes mimic the biochemical and morphological properties of the neural ECM, indicating a potential platform for neural tissue engineering.[110]

5.3. Multidomain Peptide

Multidomain peptides (MDPs), depicted in Figure 8, are composed of alternating hydrophilic and hydrophobic amino acids in the center (usually serine and leucine, SL) and charged amino acids on the 2 wings. MDPs assemble into short fibers in deionized water due to terminal charge repulsion. However, in the presence of multivalent ions, such as phosphate buffer, this class of peptides can self-assemble into longer fibers, allowing for molecular entanglement and hydrogel formation.[111]

Ma et al. functionalized VEGF mimetic peptides on MDPs and allowed them to assemble into hydrogels. The modified MDP hydrogels displayed high cytocompatibility for primary rat neurons and increased the density of new blood vessels in the injured brain. It also reduced neuronal loss at the injury site, indicating that this angiogenic MDP hydrogel is a promising treatment for neural and ischemic injury.[98]

Another example of a modified MDP hydrogel was conducted by Sarkar et al., demonstrated in **Figure 10**a–c. The MDP hydrogel was decorated with a neuroprotective peptide derived from ependymin, also known as CMX-9236 (KKDGDGDFAIDAPE). Ependymin, a glycoprotein component of the extracellular fluid, is a neuroprotective protein.[112] The in vitro data suggested that the functional MDP hydrogel reduced extracellular glutamate toxicity and that neurons harvested from cortices were able to survive, extend axons, and produce branches. The in vivo study further proved that this MDP hydrogel reduced acute injury such as cortical atrophy and ameliorated chronic injury such as neurotoxic hyperphosphorylation of tau protein. With long-term investigation and functional assessment, the authors plan to put this MDP hydrogel into the clinic.[113]

5.4. Enhancing Factor C (EF-C)

EF-C is an amphiphilic peptide sequence consisting of 12 amino acids derived from the HIV envelope protein gp 120. When dissolved in polar solvents, such as water or cell culture media, EF-C self-assembles into nanofibers with an average length of hundred nanometers and a diameter of 3 nm. EF-C nanofibers are polycationic and exhibit a positive zeta potential under physiological conditions, resulting in electrostatic interactions with the negatively charged cell membrane.[114] Additionally, these SAPs can easily be conjugated with fluorophores and co-assembled with unmodified SAP as a fluorescent probe without sacrificing their function.[115]

Our group developed an EF-C-derived SAP library with most of the peptide sequences consisting of amphiphilic 6 amino acid sequences such as KIKIQI or KFKFQF, demonstrated in Figure 8. We screened and analyzed the physicochemical properties of 27 different peptides and correlated them with their bioactivity on DRG neurons: neuron number, neurite length, and neurite branching. Our data suggested that EF-C derived SAPs possessing the highest bioactivity regarding neuronal growth stimulation have 1) a strong tendency to form a fiber structure, 2) a positive net charge with a pattern of alternating hydrophobic and positively charged amino acids, 3) higher intermolecular β-sheet content, and 4) larger cross-sectional diameter in single-fiber atomic force microscopy analysis, as depicted in **Figure 11**a,b. For visualization, SAPs were fluorescently labeled, and images of the material and cell localization were captured using microscopy, shown in Figure 11c. The selected SAPs were further investigated in a facial nerve injury model. The SAPs adhered at the lesion site for up to 3 weeks after injury which is an important prerequisite for providing long-term bioactivity, depicted in Figure 11e–i. After analyzing the functional recovery of mice treated with EF-C-derived SAPs, we concluded that the EF-C-derived SAPs alone facilitated neural regrowth in a PNI model. Further, we anticipate that EF-C-derived SAPs can play a role as a multifaceted platform for presenting multiple growth factors after fine-tuning and chemical modification.[43]

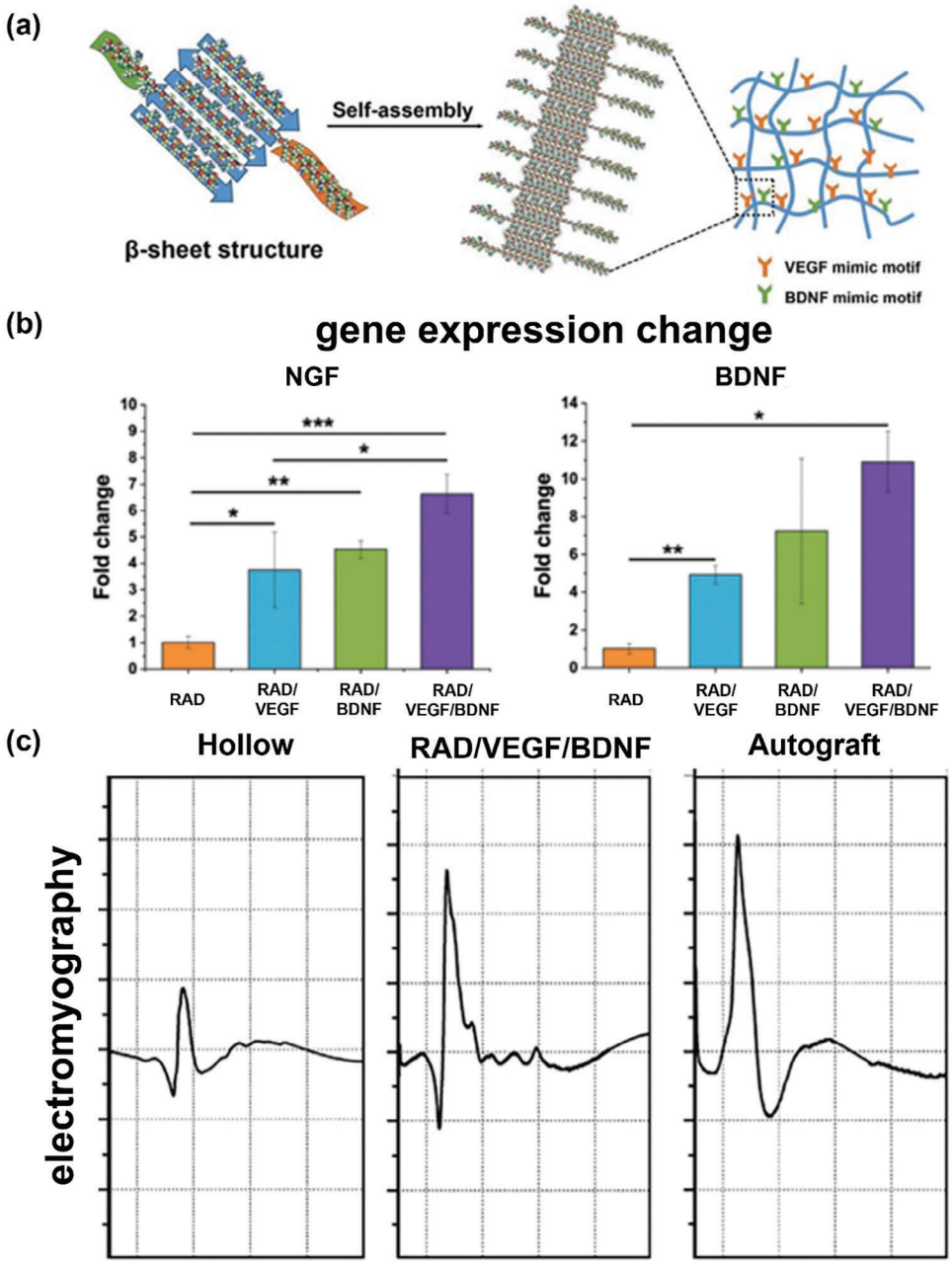

Figure 9. An example of RADA SAP for PNI repair. a) The molecular model of RADA SAP decorated with VEGF and BDNF mimetic peptides. b) Analysis of quantitative real-time polymerase chain reaction of NGF and BDNF mRNA abundance after 7 days of cultivating rat SCs on different SAPs. The increase in NGF and BDNF gene expression indicates the maturation and myelination of the SCs. c) Motor functional recovery analysis (electromyography) of an in vivo PNI model showed the therapeutic effect of different SAPs after 12 weeks of implantation. Adapted with permission.[103] Copyright 2019, Royal Society of Chemistry.

5.5. Other SAP Backbones

Yaguchi and colleagues developed a cell-adhesive fiber-forming SAP hydrogel for the treatment of brain injury. The full sequence is RIDARMRADIR. The sequence has an amphiphilic core, $AX^1X^2X^3A$, with polar amino acids at X^1 and X^3 and a nonpolar amino acid at X^2, shown in Figure 8. The jigsaw-shaped hydrophobic surface was achieved with the sequence I–A–M–A–I, which mimics the dovetail-packing domain of glycophorin A. The jigsaw-shaped hydrophobic surface allowed efficient encapsulation and sustained release of full-length VEGF. An in vitro study showed that hUVECs treated with a VEGF-loaded SAP hydrogel were prone to form a lumen-like network. The authors performed an in vivo ischemic stroke model to verify

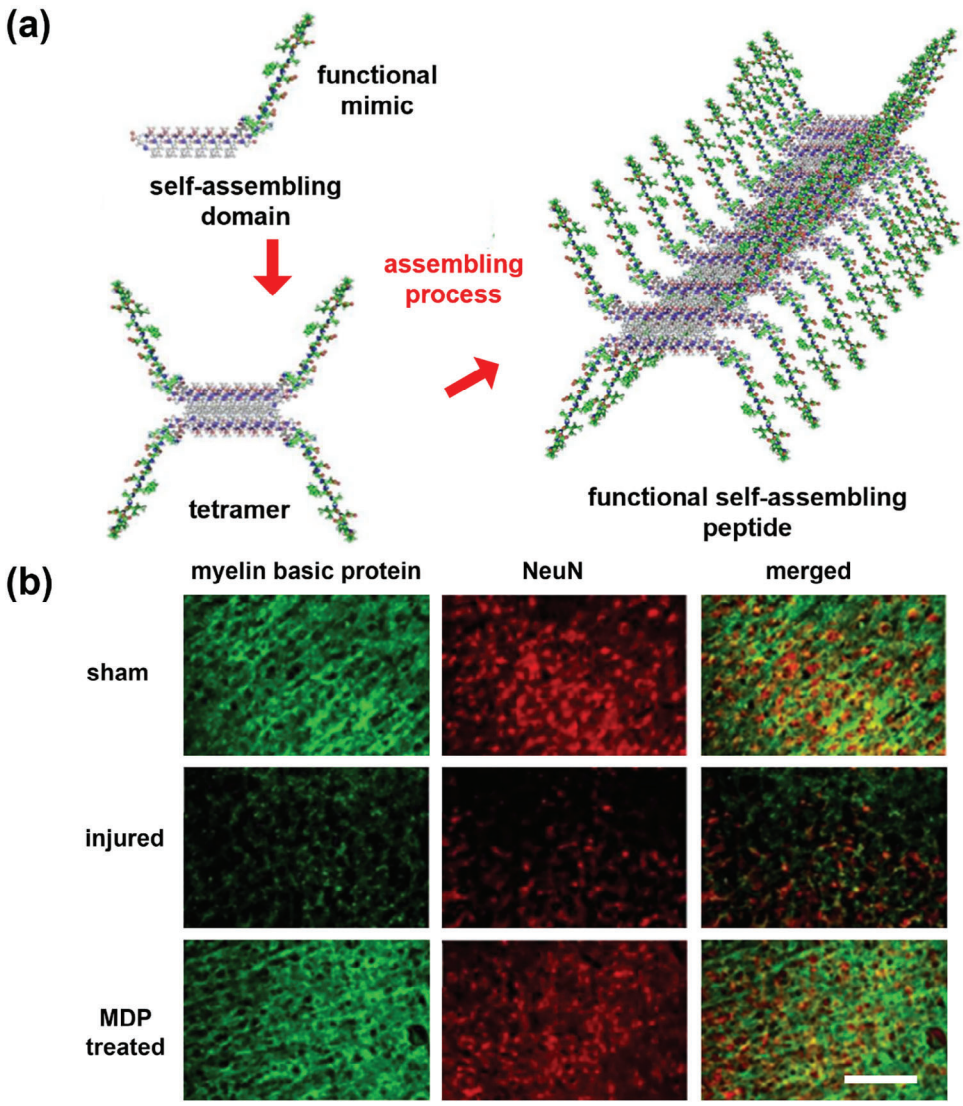

Figure 10. An example of MDP SAP for TBI repair. a) The molecular model of MDP SAP and its assembly. b) Immunostaining of brain sections of rats with antibodies directed against myelin basic protein, NeuN, and merged images in sham (the upper 3 images), injured model (the middle 3 images), and MDP SAP treated model (the lower 3 images). All images are with a scale bar of 200 µm. Adapted with permission.[113] Copyright 2021, Elsevier.

the therapeutic effect of the hydrogel, and the results demonstrated that this VEGF-loaded hydrogel promoted angiogenesis and neuroprotection.[117]

ECM protein mimetic peptides, e.g. collagen-like peptides (CLP), elastin mimetic peptides (ELP), etc., aim to resemble the properties and functions of natural and large ECM proteins.[118] CLPs are composed of triple helix bundles with each strand consisting of "G-X-Y" tripeptide repeats, where X and Y are often proline and hydroxyproline.[117] CLP SAPs can assemble into higher-order structures and typically serve as bioactive domains and physical crosslinking agents in hydrogels for tissue engineering.[119] ELPs consist of a pentapeptide repeating unit, Val-Pro-Xa-Ya-Gly, where Xa is either glycine or alanine and Ya can be any amino acid other than proline, shown in Figure 8. ELP SAPs can undergo reversible and temperature-dependent self-assembly in aqueous solutions.[120] Nelson and coauthors summarized and highlighted some recent research using ELP SAPs for neural repair.[121] Generally, ECM mimetic peptides promote cell adhesion for various cell types. However, the structural complexity of ECM mimetic peptides affects the accessibility for cell-peptide recognition.[118] More comprehensive and detailed investigations on the structure-biofunction relationship on ECM mimetic peptides are encouraged in order to advance the class of materials in the field of neural regeneration.

Other than the peptide backbones that can be synthesized using automated synthesizers, which consist of only amino acids. This allows material scientists to rapidly modify the peptide sequence in a precise manner creating peptide libraries and enabling studying sequence-structure-property correlations. The following 2 sections introduce peptide amphiphiles: PAs and aromatic peptide amphiphiles. These 2 classes of materials, unlike pure amino acids backbone, may require additional manual

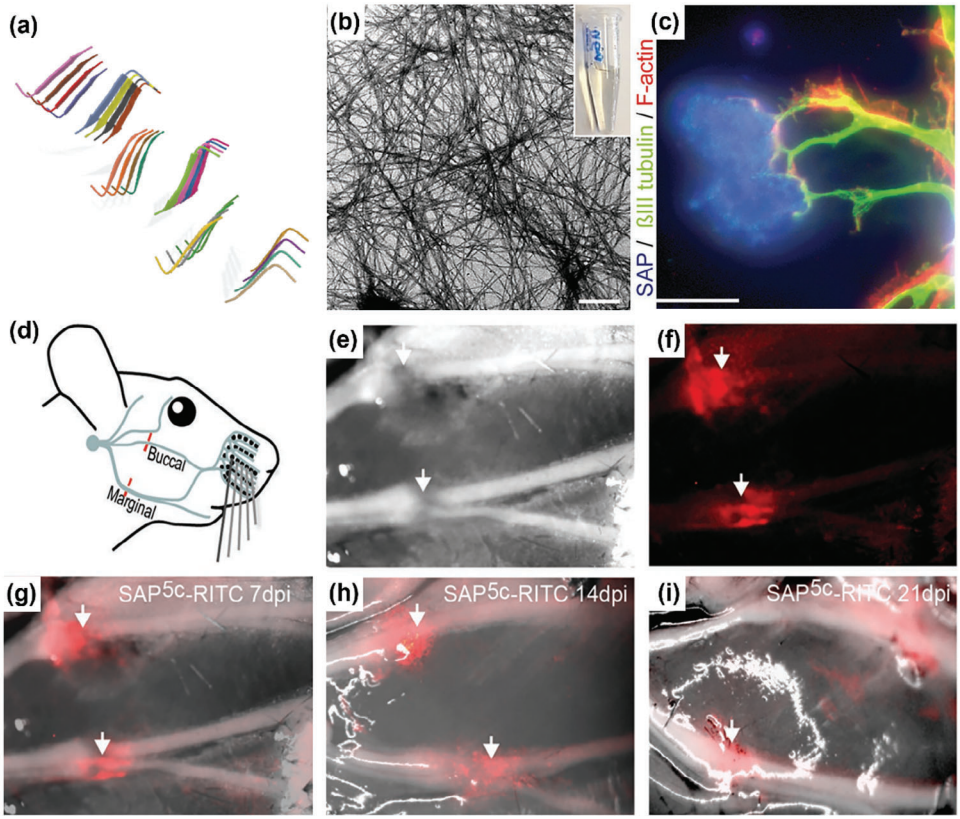

Figure 11. An example of EF-C-derived SAPs for PNI repair. a) A 3D molecular model of EF-C derived SAP, CKFKFQF is visualized by Protein Data Bank (PDB: 8OKR).[116] b) A TEM image of nanofibers assembled from EF-C monomer (scale bar = 600 nm). c) A fluorescent image of fluorophore-labeled EF-C derived SAP and cell adhesion points, stained with βIII-tubulin and F-actin (scale bar = 20 µm). d) A schematic depicting the mouse facial nerve (blue) and lesion sites (red lines) at the buccal and marginal branch e–i) Microscopic images of the lesion sites and EF-C SAP injection are indicated by arrows. Images of panels (e) and (f) were captured upon the injection, and images of panels (g) to panels (i) were recorded at 7, 14, and 21 post-days post-injury (dpi). Adapted with permission.[43] Copyright 2019, WILEY-VCH GmbH.

synthesis. They consist of amino acids and hydrophobic motifs, such as aliphatic chains, aromatic groups, or heterocyclic compounds. These compounds introduce non-natural compounds into the self-assembled structure and additional functionalities.

5.6. Peptide Amphiphile (PA)

PA molecules were first reported in the Stupp laboratory in the early 2000s. They self-assemble into long nanofibers and form hydrogel biomaterials with high water content that mimic the ECM. Fiber-forming PA molecules, depicted in Figure 8, are comprised of an aliphatic tail with more than 10 carbon atoms, a peptide sequence with a high tendency to form β-sheets, a charged peptide sequence to increase hydrophilicity, followed by a bioactive segment.[120] The length of the hydrophobic tail, the sequence of the structure-forming peptide, and the additional spacers between each segment of PA molecules play important roles in the physicochemical properties and the final self-assembled structure and bioactivity.[77,122,123]

Okur et al. selected an NGF sequence that was coupled to PA molecules. The NGF-binding PA nanofibers stimulated the differentiation of PC12 cells into neuron-like cells and the expression of neuronal markers, such as βIII-tubulin. In addition, the NGF-binding PA nanofibers also supported the survival of sensory DRG neurons and induced axon outgrowth and synaptic connectivity for DRG neurons.[124]

Netrin-1 is a chemotropic factor responsible for axon growth, guidance, and synaptogenesis. Netrin-1 mimic was synthesized on PA molecules and tested on primary neuronal culture, shown in **Figure 12**a–c. Conjugated to PA molecules, only cyclic Netrin-1 mimetic PA nanofibers (N1-PA) efficiently activated the receptor and associated signaling pathway in primary mouse cortical neurons. These findings suggest that the Netrin-1 mimetic PA nanofibers can be a potential therapy for SCI repair as well as for diseases or injuries associated with netrin-1 deficiency, such as stroke and Alzheimer's disease.[125]

5.7. Aromatic Peptide Amphiphile

Proteins carry an enormous amount of structural and functional information. The design of aromatic peptide amphiphiles aims to provide a minimalistic and scalable class of materials as an alternative to natural proteins.[126] The general structure of aromatic peptide amphiphiles consists of an aromatic group, a linker, a

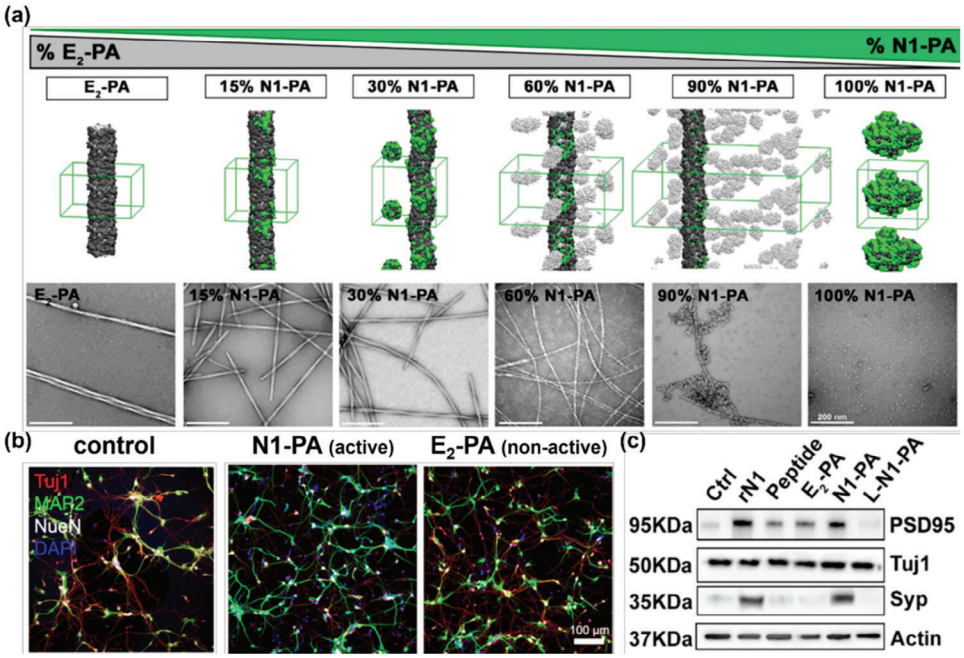

Figure 12. An example of PA SAP for neural repair. a) Coarse-grained molecular dynamics (CG-MD) simulations suggested that 60% or less of the co-assembling ratio of Netrin-1 decorated PA monomer and PA monomer to obtain fiber structure. Meanwhile, the corresponding TEM images of different co-assembly ratios were taken to confirm the results of the CG-MD simulation. b) Representative confocal microscopy images of primary neurons after being treated with a poly d-lysine coating (control), pure PA (E_2-PA), and N1-PA for one week. c) Representative Western blot of PSD95, Tuj1, and synaptophysin (Syp) in primary neurons after treatment with poly d-lysine coating, recombinant netrin-1 (rN1, the positive control), netrin-1 mimetic peptide (Peptide), E_2-PA, PA and N1-PA, and linear 15% netrin-1 mimetic peptide modified PA (L-N1-PA) for 2 weeks. Adapted with permission.[125] Copyright 2023, American Chemical Society.

functional peptide sequence, and the C-terminus, depicted in Figure 8. The aromatic moiety triggers the self-assembly via directional aromatic stacking, also known as π-π stacking, with some commonly used aromatic groups being the derivatives of fluorene, phenyl, naphthalene (Nap), pyrene, etc.[126,127] The N-terminal capping group can be functionalized with heterocyclic capping groups to introduce more functionality; for example, redox-responsive groups and fluorophores.[127,128] The length and chain flexibility of a linker group affect the conformation and the chirality of the assembled structures, and a more linear linker is preferable to allow effective intermolecular aromatic stacking. In a functional peptide sequence, the amino acids also have a significant influence on the assembled structures. For example, the side chain of the amino acids may introduce aromatic groups (F, Y), hydrophobic moieties (V, L), and charged residues (E, D, H, R, and K). The C-terminus, like the carboxylic group, amide group, and methoxycarbonyl group, regulates the self-assembled structures via the hydrophilicity and charges. Moreover, the C-terminus can be further chemically modified to tune the self-assembled structures or introduce additional functions.[126]

The Fmoc protecting groups are ubiquitous in aromatic-modified peptides which form stiff hydrogels with fibrillar nanostructures.[126] Roy's lab co-assembled 2 laminin mimetic peptides, Fmoc IKVAV, and Fmoc YIGSR, creating a self-sorted fiber network in a single hydrogel scaffold. The non-covalent entanglement of the 2 laminin mimetic peptides provided a neurotrophic niche for C6 glioma cells and SH-SY5Y neuroblastoma cells, which was confirmed by proliferation assay and neurite outgrowth experiments.[92] James et al. reported thiophene-modified peptide sequences that self-assembled into nanofiber hydrogels as an example of heterocyclic end-capping groups that can induce self-assembly and introduce conductivity to an SAP system. Despite the successful synthesis and characterization, further biological evaluation is necessary before considering this conductive nanofiber hydrogel as a therapeutic treatment for neural tissue engineering.[129] Another example of heterocyclic end-capping groups triggering self-assembly and conferring conductivity to peptide sequences was published by Arioz and colleagues. Tetra(aniline)-modified peptides self-assembled into conductive nanofiber hydrogels to stimulate the differentiation of PC12 cells. Further in vivo studies along with a thorough investigation of the mechanism of neural differentiation will pave the way for the application of this conductive SAP hydrogel for neural regeneration.[88]

Interestingly, despite the significant differences in sequence pattern, length, composition, and origin (natural vs designed), the above-mentioned classes of peptide materials show remarkable similarities in their ability to support neuronal growth. All these peptides tend to form highly anisotropic structures, e.g. nanofibers or fiber bundles, which resemble the morphology of the fibrous ECM proteins. Minimal peptides such as the aromatic peptide amphiphiles are particularly cost-effective to produce but do not show a robust assembly under varying conditions. In contrast, longer peptides such as the RADA family or PAs with longer alkyl chains have the advantage of forming nanofibers in a variety of buffers and supporting a wide range

of bioactive epitopes that can be coupled to the peptide without altering the resulting assembled structure. When it comes to designing peptide materials for neural regeneration, an optimal material does not (yet) exist and the pros and cons of each class of materials need to be considered.

6. Summary and Outlook

In this review, we introduced mechanisms and model systems of PNI, SCI, and TBI. In addition, we discussed the challenges associated with neural self-repair for each type of injury. Some current clinical treatments include neuroprotective drugs and surgical procedures, which target the primary lesion. Here, synthetic peptides can offer interesting new avenues for new treatment approaches, but more research into improving their performance and understanding the interactions between cells and materials is needed.

It is important to consider the fundamentals of cellular biology before delving into in vivo models and clinical translation studies. Therefore, we provide an overview of commonly used cell lines (PC12, Neuro2a, and SH-SY5Y), stem cells (PSCs, MSCs, and NSCs), primary cells, and ex vivo explant cultures, as well as their respective advantages and disadvantages in terms of clinical relevance. However, neuronal repair involves multicellular interactions, so investigating the co-culture of different cell types will facilitate a better understanding of the mechanisms of neural regeneration and the ability to mimic the complex environment. In comparison to natural biomaterials or synthetic polymers, synthetic peptides offer improved stability and bioactivity for neural repair, respectively. We discuss 2 main categories of the applications of therapeutic peptides, as drugs and SAPs as neural-specific ECM mimics. To broaden the usage of soluble peptides from the perspective of chemists, increasing the metabolic stability by using non-natural amino acids or D-form amino acids, which are less prone to hydrolysis, is one possibility. Another method to increase the stability of soluble peptides is to combine them with polymeric materials such as PEG. To enhance the delivery efficiency of small molecule or peptide drugs, a strategy can be to couple them via chemical-responsive groups, such as matrix metalloproteinases or reactive oxygen species-responsive linkers.

For SAPs serving as ECM mimics, their degradation within the body depends on their peptide sequence and assembled structure with amyloid-type assemblies being highly stable. However, the mechanical stability of pure peptide scaffolds often is not sufficient to serve as long-lasting cell scaffolds. One possible avenue to address this is by combining SAPs with covalent polymers as reinforcement. Such hybrid materials may also reduce the production cost associated with peptide synthesis and address issues of scalability. Translational concerns associated with the use of SAPs in nerve regeneration include 1) the difficulty of controlling the self-assembly of peptides over multiple length scales and 2) the uncertainty if SAPs modified with epitopes or growth factor mimics have to be considered as medical devices or pharmacological products. Depending on the category they are assigned to, different evaluation procedures may be mandated by the authorities. To overcome these hurdles, collaborative projects between material scientists, biologists, chemists, and clinicians are necessary to develop methods to control the resulting SAP assemblies in a spatially and temporally controlled manner. Promising approaches for controlling the macroscopic alignment of SAP scaffolds include shear-induced or magnetically controlled orientation. A strong collaboration between biologists and chemists is needed to establish high throughput methods for faster and more accurate biological readouts, thus creating structure-activity correlations. Collaborative works among biologists, material scientists, and chemists are critical to clarify the main therapeutic effect of SAPs on neural cells as evidence to proceed to clinical trials.

We believe that building ECM mimics based on SAPs brings enormous potential for neural regeneration. In theory, SAPs can introduce physical stimuli (matrix stiffness, topography, molecular arrangement, co-assembling, and conductivity) and neurotrophic motifs (epitopes and growth factor mimics) to neural cells at the same time. In addition, the possibility of locally injecting the SAP into the injury site may significantly reduce the complexity of treating neuronal injuries. Having the possibility to combine both physical stimuli and bioactive motifs, computational chemists and material scientists can apply machine learning and computer simulations to reduce trial and error and speed up the development of neural regeneration. Combination therapy of biomaterial and non-invasion electrical stimuli, e.g. a conductive SAP may be employed to direct and target electrical impulses to regenerate nerves, which might create new therapeutic opportunities. In the best case, surgeons are involved in the development of new materials, which are easy to store and handle, to truly meet the clinical needs of patients.

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Conflict of Interest

The authors declare no conflict of interest.

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extracellular matrix, matrix property, neural regeneration, peptide, self-assembly

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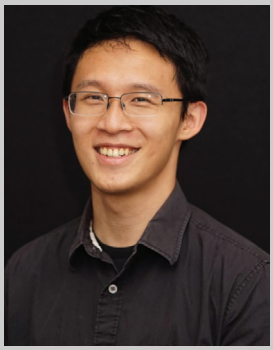

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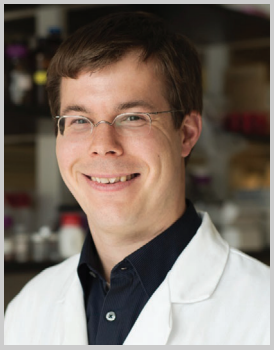

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