CHAPTER 17

Tissue engineering of the nervous system

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17.1 Learning objectives

After reading this chapter you will be able to:

- Appreciate how the regenerative potential of the mature peripheral nervous system (PNS) contrasts with the more limited capacity for regeneration in the mature central nervous system (CNS).
- Appreciate the critical gap length in peripheral nerves, and how different tissue engineering strategies are being developed to successfully bridge large nerve defects.
- Recognize there are different types and stages of spinal cord injury (SCI) and how this affects potential treatments.
- Understand that scar formation following injury to the adult CNS presents a key challenge for circuit repair and appreciate the importance of influencing the associated immune and inflammatory responses for successful regeneration.
- Appreciate that different therapeutic approaches are needed when treating traumatic versus degenerative brain pathologies, and note the unique nature of the optic nerve as a model for CNS regeneration.

When peripheral nerve segments were used as "bridges" between the medulla and spinal cord, axons from neurons at both these levels grew approximately 30 millimeters.

S. David and A. J. Aguayo, 1981

One man's "magic" is another man's engineering.

R. A Heinlein, 1973

Tissue Engineering. https://doi.org/10.1016/B978-0-12-824459-3.00017-2

17.2 Introduction

Diseases or injuries to the nervous system are devastating to the individual and have substantial societal implications and costs. The **PNS** and **CNS**—spinal cord, brain, and retina—have different molecular and cellular environments and, therefore, require specific tissue engineering repair strategies. For example, compared to the PNS, there is more limited plasticity and regrowth capacity in the CNS, due to inhibitory environmental factors that suppress growth as well as intrinsic changes to the regenerative capability of injured mature neurons. The primary focus of tissue engineering in the PNS or CNS is the regeneration and reconnection of severed axons that have been isolated from their targets. In the PNS, this often requires the implantation of artificial structures to bridge nerve defects in order to support and guide regenerating axons. Depending on the **neuropathological condition** under investigation, injectable matrices for the CNS can provide a substrate for axonal growth in the inhibitory CNS environment, and act as a delivery vehicle and/or provide a favorable environment for repair-supporting cells. Because of a long history of bridging studies in the PNS, this system is discussed first, followed by a description of tissue engineering strategies that are being developed for CNS repair.

17.3 Peripheral nerve

Peripheral nerve injuries (PNIs) can lead to lifetime loss of function and disfigurement even though a **peripheral nerve** (PN) is capable of a substantial amount of regeneration when crushed or severed. PN contains only the **axon** part of each contributing neuron and one could consider the peripheral nerve trunk as a protective structure for axons. The cell bodies of **sensory neurons** that give rise to PN axons are located in structures adjacent to the spinal cord (dorsal root ganglion (DRG)) or in cranial ganglia, while the cell bodies of **alpha motor neurons** that provide axons to innervate and drive skeletal muscle contraction are located within the CNS (brainstem or spinal cord gray matter). **Gamma motor neurons** are a different type of cell that also have their cell bodies in the spinal cord, but whose axon has a smaller diameter compared to alpha neurons. Gamma neurons do not innervate muscle fibers involved in contraction but contact sensory receptors called muscle spindles, thereby playing a critical role in monitoring and adjusting sensitivity to muscle length and stretch. Ideally, successful PN repair should involve all these neuronal classes. Once a PN defect is bridged, regenerating axons have demonstrated guidance over long distances within the degenerated distal nerve stump, along naturally occurring conduits called bands of Büngner (see **Bands of Büngner**).

17.3.1 Peripheral nerve anatomy

Peripheral spinal nerves (Fig. 17.1) are formed from dorsal or ventral roots of the spinal cord. Dorsal roots contain sensory (or afferent) axons of the somatic and **autonomic nervous systems** which transmit signals into the CNS from the periphery (skin, muscles, joints, etc.). Ventral roots contain motor (or efferent) axons which transmit motor signals from CNS-originating neurons out to muscles and glands. Fig. 17.1a shows schematically drawn cell bodies of sensory neurons (blue) and alpha motor neurons (red) and their corresponding nerve processes. Motor commands typically originate in the brain (cortex, midbrain, and brainstem) and contact lower motor neurons in the brainstem motor nuclei or ventral spinal cord, sometimes directly but more commonly via interneurons located within the gray matter. In addition, there are twelve **cranial nerves** (CNs), the majority with nuclei located in the brainstem. CN I (the olfactory nerve) and CN II (optic nerve) are considered part of the CNS, while CN III–CN XII are in the PNS. Individual CNs can be purely sensory or motor or may contain both types of axons.

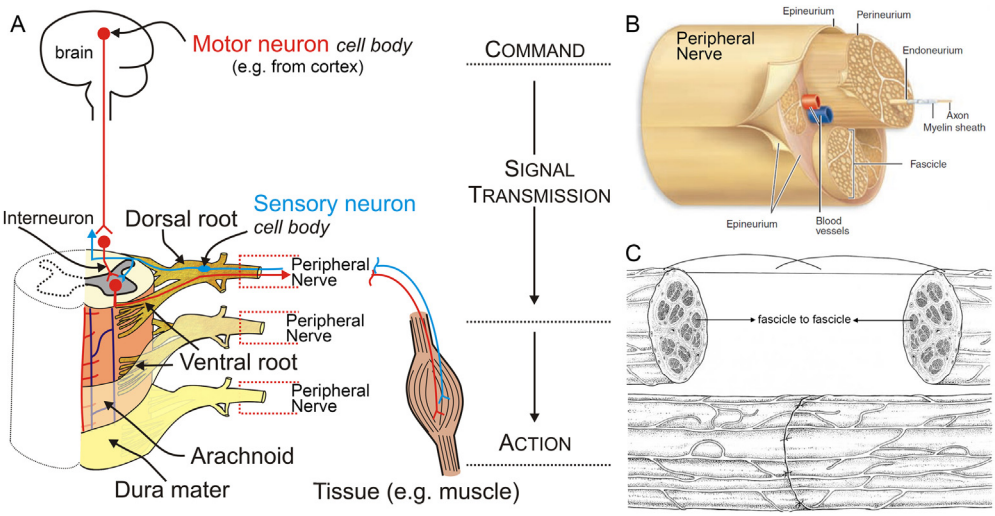

FIGURE 17.1

(a) Anatomical overview of the peripheral nerve and connections to the central nervous system. (b) Ultrastructure of the peripheral nerve, (c) Fascicle-to-fascicle alignment during microsurgical reattachment using sutures in a tensionless manner. *(a) From Dalton, P.D., et al., 2008. Tissue engineering of the nervous system, In: Van Blitterswijk, C., et al. (Eds.), Tissue Engineering. Academic Press, pp. 611—647. (b) From Mescher, A.L., 2013. Junqueira's Basic Histology, 13th ed. McGraw-Hill. (c) From Lundborg, G., A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg (0363-5023 (Print)).*

PNs are discrete fibro-collagenous trunks (Fig. 17.1b) filled with sensory and motor axons and include Schwann cells, perineurial cells, and fibroblasts. Due to limb movements and the resulting tensile and compressive stresses, the outermost sheath, the epineurium, provides a protective structure to the axons. This fibro-collagenous epineurium binds individual axon-filled fascicles into one nerve trunk (Fig. 17.1b). Many (but not all) of the axons are myelinated by supporting glial cells called Schwann cells. Smaller diameter, nonmyelinated axons are embedded within invaginations of Schwann cells forming so-called "Remak bundles."

17.3.2 Peripheral nerve injury

Regeneration of severed axons requires that the parent cell bodies survive the initial trauma. After injury to a PN, **retrograde degeneration** in the nerve stump occurs back to the node of Ranvier (see Section 17.14) that is located most proximal to the lesion. It is important to note that axonal injuries that are closer to the spinal cord or brainstem are more likely to cause retrograde neuronal cell death and are more complex than injuries that are located in more distal PN. After the initial PNI, the **proximal** nerve stump will swell, but undergoes minimal damage compared to axons in the **distal** stump. After a nerve injury, the distal axons degenerate by a process termed **Wallerian degeneration** (in both PNS and CNS, a sequence of active degenerative/reactive events in the distal axon); changes in PN include axonal cytoskeleton breakdown and shedding of myelin lipids by Schwann cells. Over several days, macrophages and Schwann cells clear **myelin** and other debris: a critical process that increases the potential for PN regeneration. Importantly, Schwann cells in the distal portion of the severed nerve proliferate and begin to secrete specific growth factors that stimulate axonal regrowth. After debris clearance, axonal sprouting and regeneration begins at the tip of the proximal stump and continues toward the distal stump.

Surgically, accurate realignment of the original fascicles is critical to increase the chances of functional recovery in microsurgical repair (Fig. 17.1c) and the ability to achieve **"fascicle-to-fascicle" repair** of large injury gaps is an important long-term goal for PN tissue engineering.

In the distal nerve stump, basal lamina tubules form Schwann cell filled bands of Büngner, which play a crucial role in successful guidance of regrowing axons to their original targets in PN regeneration.[1] Axonal growth in humans occurs at a rate of about 1 mm/day and appears to be more effective in younger people. Significant injuries closer to the CNS therefore can take many months to heal; not only must axons regenerate and reestablish the correct connections, but remyelination needs to occur and also maturation of synapses is required. This prolonged period of denervation has deleterious effects on the Schwann cells within the distal nerve stump and on target tissues, making it important that regeneration occurs as efficiently and as quickly as possible.

Anatomical nomenclature

Rostral or Anterior = Head or front end.
Caudal or Posterior = Tail or hind end.
Proximal = The (injured) part of the tissue closer to the neuron cell body.
Distal = The (injured) part of the tissue away from the neuron cell body.
Lateral = Away from the midline.
Medial = Toward the midline.
Dorsal = Back/top side.
Ventral = Belly/bottom side.

17.3.3 Autologous nerve grafts (autograft)

PNI most commonly results from blunt trauma such as crush injury or from penetrating injuries caused by sharp instruments, but it is also associated with fractures and fracture dislocations. Crush injuries are therefore more common than nerve transections. When nerve stumps are unable to be surgically reattached using sutures in a tensionless manner, a bridging section of an implanted autologous nerve is often used, and two end-to-end sutures are performed. The damaged tissue is replaced by a nerve taken from another (less critical) site, for example, the purely sensory sural nerve.[2] However, this also results in some **donor site morbidity** and loss of sensation to the lateral heel/foot region. The inability to match the fascicular pattern of the donor nerve to that of the damaged host nerve may lead to some degree of misrouting of regenerating axons in which sensory axons may enter and follow the original trajectory previously occupied by motor axons (and vice versa). Despite great care being taken during microsurgical repair, axonal misdirection is a frequent problem. Furthermore, donor nerves are often of small caliber and limited in number. Although the PN autograft is regarded as the "gold standard" to which all alternative therapies are compared, the above-mentioned problems have driven the search for tissue engineering alternatives to this treatment.

17.3.4 Use of nerve guides (tubes) in the lesioned PNS

An off-the-shelf alternative for injury-induced gaps of PN (typically less than 10 mm but up to 70 mm) are **nerve guides** which function by acting as a conduit between the severed proximal and

distal nerve stumps. Hollow tubes (nerve guides or nerve conduits) have successful outcomes that are comparable to the autograft in certain clinical situations. Fig. 17.2 schematically shows the general sequence of regeneration. After implantation, fibrin from damaged blood vessels and cytokines (including neurotrophic factors) primarily generated by the Schwann cells builds up within the nerve guide (Fig. 17.2a). Within 7 days, an oriented fibrin scaffold is formed, and cells from the perineurium penetrate into the nerve guide, while axonal debris is removed by Schwann cells and macrophages in the distal stump (Fig. 17.2b). From 7 to 14 days, migrating endothelial cells, Schwann cells, and regenerating axons penetrate into the nerve guide using the newly formed fibrin scaffold as a substrate (Fig. 17.2c). Between 14 and 56 days, Schwann cells remyelinate the regenerating larger diameter axons that exit the nerve guide, directed by the Bands of Büngner in the distal nerve stump to their targets.

Currently, there are several nerve guides on the medical device market, as shown in Table 17.1. Their performance is reviewed in depth by Deumens et al.,[2]. All of these hollow nerve guides are biodegradable and provide a protective semipermeable membrane which separates the injury site from surrounding tissues and through which nerve regeneration and tissue repair are supported. Current research focuses on filling the lumen of nerve guides with matrices, scaffolds, cells, and/or drug delivering therapies to increase the efficiency of tissue repair.[3,4]

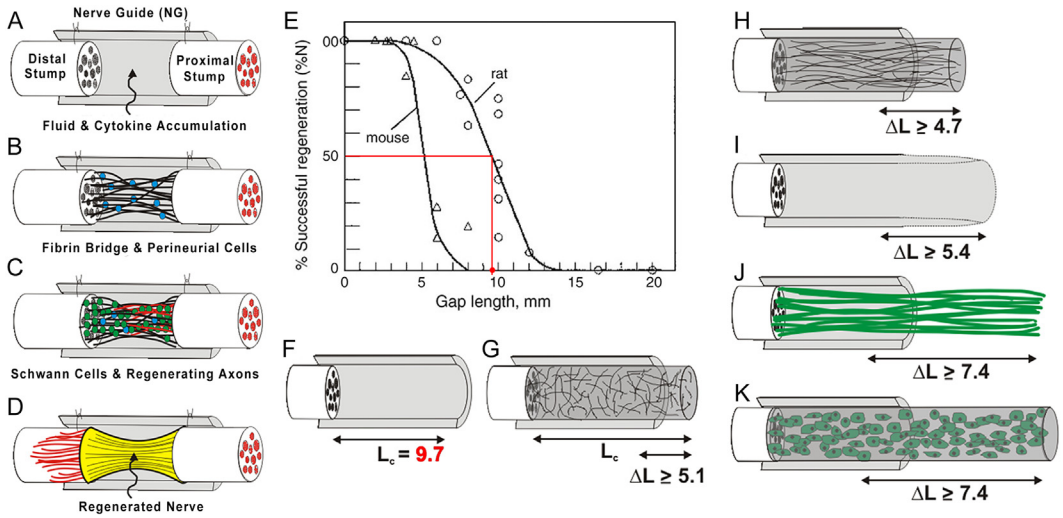

FIGURE 17.2

Progression of regeneration within a nerve guide: (a) hours, (b) days, (c) weeks, and (d) months after injury. (e) Critical gap defect (Lc) and regenerative capability. An empty nerve guide (f) increases (g) its Lc value with (h) oriented matrices, (i) degrading polymers, (j) fiber inclusion, or (k) Schwann cell transplantation. *(e) From Zhang, M., Yannas, I.V., 2005. Peripheral nerve regeneration. Adv Biochem Eng Biotechnol. 94, 67–89.*

Bands of Büngner

Although the critical gap length (Lc) for humans is approximately 30 mm, once the regenerating axons bridge this defect, there is a natural scaffold prepared in the distal segment of the peripheral nerve. Axons will travel distances well above the critical gap length along Schwann cell—containing tubular basal lamina, which are termed "Bands of Büngner" after the German neurologist Otto von Büngner (1858—1905) and are often described as "Schwann cell columns." After PNI, the cytoskeleton of the severed axon is degraded and Schwann cells, which were previously wrapped around the axons, dispense with their myelin sheath and clear debris, adopting a repair phenotype. The Schwann cells partly clear the myelin while increasing numbers of macrophages in the distal stump complete the task. Throughout such Wallerian degeneration, the basal lamina tubes that support these cells remain and act as specific tracts that channel the regenerating axons to the final targets.

With the development and optimization of microsurgical techniques for peripheral nerve repair, the success of end-to-end suturing greatly depends on the alignment of the fascicles. Therefore, the penetration of a sensory axon down a basal lamina tube previously filled with an axon from a motor neuron (and vice versa) is an ineffective (but frequent) result. Nevertheless, the bands of Büngner are the naturally occurring substrate for regeneration over long distances distal to the PN injury.

17.3.5 Critical gap length

A **critical gap length** (L_C)[5] is an indication of the limits of PN regeneration using nerve guides. It is the distance between transected nerves where the regeneration success is approximately 50% and varies depending on species.[6] When the transected PN length approaches this experimentally determined length, the chances of successful regeneration within nerve guides are diminished. Zhang and Yannas have shown the frequency of successful regeneration (%N) within rat and murine models using silicone nerve guides and L_C (when $\%N = 50$) has been determined to be 9.7 ± 1.8 mm for rats and 5.4 ± 1.0 mm for mice.[5,7] The introduction of matrices, fibers, and cells within nerve guides likely increases the L_C to varying degrees, which is an important measure of therapeutic success. The change in length (ΔL) due to a therapy is the difference between the L_C of the experimental device and the L_C of the control group. The introduction of a therapy will generally increase the L_C; Fig. 17.2. shows schematics based upon this approach of assessing additional therapies. Using the L_C normalization of therapies in the PNS, the therapies that significantly increased ΔL were analyzed and regenerative theories put forward.[7]

17.3.6 Nerve guides as supports for regeneration strategies

Nerve guides are often filled with a therapeutic agent to improve the Lc. Matrices and scaffolds for the PN aim to mimic an ECM environment that promotes increased regeneration after injury, while cellular grafts and neurotropic factors provide an improved **chemotactic** environment after injury.

Matrices

Currently, no ideal matrix promotes the regeneration in a nerve guide that is better than the level achieved using a PN autograft[3]; however, the L_C generally increases for nerve guides when they contain matrices within the lumen. Effective matrices have similar mechanical properties in that they are all soft, viscoelastic hydrogels with high water content; however, they cannot be too concentrated (or dense) as

this would lead to obstruction of cell and axonal penetration. While matrices are formulated into **bioinks**[8] for biofabrication approaches, these require the capacity to be translated to the clinic. It is important to mention that when the nerve guide is sutured into position, a fibrin bridge spontaneously forms due to the secretion of factors from the proximal and distal stumps. This natural process has been taken advantage of in order to engineer matrices that fill the lumen of nerve guides.

Oriented matrices

In vivo experiments with oriented matrices have a higher L_C value than nonoriented matrices of the same material (Fig. 17.2h). This was shown with fibrin and collagen, which can be aligned with a magnetic field during gelation due to the fact they are diamagnetic macromolecules.[9] Oriented substratum and techniques to form such structures on a relevant scale are therefore important issues in neural TE. Axons will regenerate significant distances along Schwann cell–containing basal lamina tubes or similar structures (see **Bands of Büngner**).

Scaffolds

The endoneurium of the PN consists of the interstices lying between the longitudinally oriented basal lamina tubes and the perineurium and is rich in highly aligned collagen fibrils (Fig. 17.3a and b). It is widely anticipated that similar channel-like morphologies would be ideal in the supporting directed growth of neurites. While a nerve guide (Fig. 17.3c) contains the severed stumps of the PN, the nerve itself has a structure based on nerve fascicles containing myelinated and nonmyelinated peripheral axons (Fig. 17.3d). Jeffries and Wang[10] produced channeled nerve guides by combining oriented electrospun fiber collection with channel templates, which could be rolled up to produce the channel structures shown in Fig. 17.3e–f. One simple technique to induce orientation involves the controlled growth of ice crystals through the hydrogel matrix to impart similar guidance structures (Fig. 17.3g). Such approaches are applicable to a range of hydrogels, essentially converting oriented materials into scaffolds with guidance properties.[11,12] The NeuroMaix nerve guide developed by Matricel GmbH is in clinical trials and is based on this uniaxial freezing approach.[13] Simple molding and construct dissolution is another approach to create guidance channels, as rendered in Fig. 17.3h.[14] For such channels, surface modification of synthetic polymers with peptide sequences such as RGDS (Arg-Gly-Asp-Ser), YIGSR (Tyr-Ile-Gly-Ser-Arg), and/or IKVAV (Ile-Lys-Val-Ala-Val) significantly increases neurite growth in vitro. Scaffolds made using biologically derived polymers such as collagen inherently contain such cues that aid in the guidance of neurites.[15]

Multiple studies using submicron diameter fibers will also guide growth cones and neurites. Fibers are attractive guidance substrates as they can be bundled together and used as oriented scaffolds (Fig. 17.3e–f). Interestingly, when oriented collagen fibers are embedded within a hydrogel, the Schwann cell will significantly stretch more than one placed in that hydrogel environment alone (Fig. 17.3h).[16] Using oriented electrospun sheets within nerve guides, a more challenging 14 mm nerve gap could be repaired.[17] There remains extensive research to perform on scaffold manufacturing technologies (see Chapter 11 **Scaffold design and fabrication**) for PNI, including structures and morphologies that better mimic fascicles. Optimal structures likely will replicate the natural fascicle structure and Fig. 17.3 highlights how scaffold design may aim to replicate this morphology.

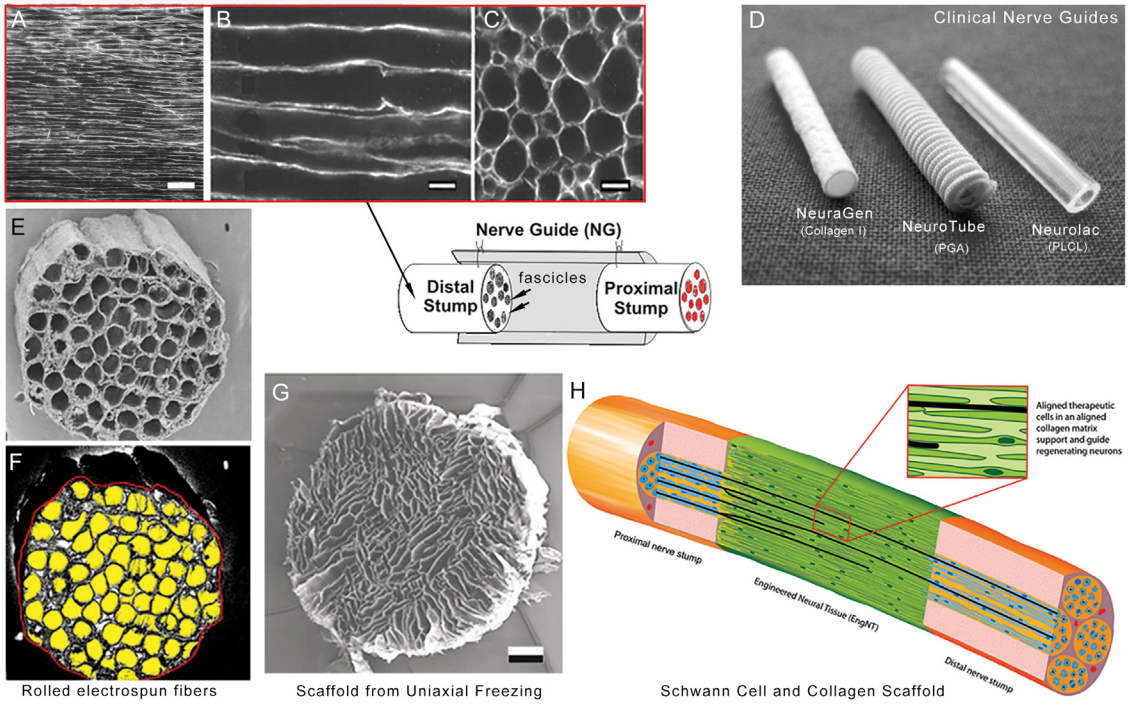

FIGURE 17.3

Strategies to mimic the peripheral nerve ECM architecture. (a-c) Natural ECM of the PN, (d) Clinical nerve guides, (e and f) Scaffold and channels fabricated with an electrospinning approach, (g) an oriented scaffold made using a freezing technique, (h) aligned collagen matrix. *(a-c) From Ceci et al., 2014. Axon-Schwann cell interactions during peripheral nerve regeneration in zebrafish larvae. Neural Dev 1749—8104. (d) From Tian et al., 2015. Strategies for regeneration of components of nervous system: scaffolds, cells and biomolecules. (e and f) From Jeffries and Wang, 2012. Biomimetic micropatterned multi-channel nerve guides by templated electrospinning. Biotechnol Bioeng 109 (6), 1571—1582. (g) From Riblett et al., 2012. Ice-templated scaffolds with microridged pores direct DRG neurite growth. (h) From Phillips, 2021. "EngNT"—Engineering live neural tissue for nerve replacement.*

Acellular allografts

As an alternative to autologous tissues and nerve guides, nonautologous sources have also been explored for bridging of PN lesions. For this purpose, both **allogenic** (same species) and even **xenogeneic** (different species) tissues have been considered. Although such allogenic and xenogeneic alternatives may be in plentiful supply, they present several risk factors including immunogenic mismatching as well as the possible transmission of disease. Immune suppression may be used to prevent graft rejection in such circumstances, but long-term immune suppression is not the ideal solution.

The treatment of allogenic nerves involves freeze-thawing of donor tissue to reduce immunogenicity by effectively killing all cellular components (e.g., SC, perineurial cells, and fibroblasts) while leaving the ECM (see Chapter 5 **Extracellular matrix as a bioscaffold for tissue engineering**), including SC basal laminae and endoneurial collagen fibrils, largely intact. Such cell-free PN grafts have been reported to

be far less immunogenic.[18] Grafts such as the FDA-approved Avance product (Table 17.1) promote successful regeneration across PN deficits of up to 30 mm.[19]

Schwann cell grafts

When cultured Schwann cells are included within nerve guides, large shift lengths (ΔL) result and significant gap lengths may be bridged (Fig. 17.2k). For example, the morphological and phenotypic characteristics of the bands of Büngner have served as inspiration for a biofabricated Schwann cell–laden implantable microtissue. These structures successfully increased the rate of axonal extension in vitro from primary rat spinal motor neurons and rat DRG sensory neurons.[20] Schwann cells can also be suspended within a matrix or seeded within decellularized PN allografts.[18] The challenge with using Schwann cell transplants is that their regenerative phenotype is usually transient.[21] For cellular nerve graft or cell/biomatrix hybrid structures, the long-term supply of **neurotrophic factors** to promote axonal regrowth may be achieved by the Schwann cells themselves, or specific factors can be obtained by targeted genetic modification of Schwann cells using viral vectors in vivo or ex vivo.[18] Depending on the type of growth factor that is introduced into grafted Schwann cells, differential effects have been reported on graft morphology, the number and type of regenerating sensory and motor axons, the extent of remyelination, and the degree of functional recovery including locomotion.[18,22–24] Schwann cell transplantation has been a primary focus for cell therapies in the treatment of PNI, although the source of the Schwann cells is another factor that needs to be addressed. Any eventual clinical solution for bridging large injury gaps will have to compete with the relatively low-cost alternative of the autograft, nerve guides, and decellularized allografts. In addition to Schwann cells, other cell types including stem cells (mesenchymal or adipose-derived) or bone marrow cells (including stromal or mononuclear cells) have been paired with a nerve prosthesis to repair **critical-sized defects** in peripheral nerves.[25]

Table 17.1 Commercially available and FDA approved nerve conduits.

Product name	Composition	Diameter	Length	Degradation time	Manufacturer
Neurotube	Poly(glycolic acid)	2–8 mm	4 cm	3 months	Synovis Micro Companies Alliance, Birmingham, AL
NeuroMatrix Neuroflex Neuromend	Type 1 collagen	2–6 mm	2.5 cm	7 months	Collagen Matrix Inc., Franklin Lakes, NJ
Neurolac	Poly(lactide-co-caprolactone)	1.5–10 mm	3 cm	16 months	Polyganics BV, Netherlands
NeuraGen	Type 1 collagen	2–7 mm	2 cm	4 years	Integra Neuroscience, Plainsboro, NJ
SaluBridge	Salubria (poly (vinyl alcohol) hydrogel)	2–10 mm	6.35 cm	No degradation	SaluMedica LLC, Atlanta, GA
Advance	Decellularized human nerve allograft	1–5 mm	7 cm	No data, but expected to resorb	Axogen Corp., Alachua, FL

Adapted from Schlosshauer, B. et al., 2006. Synthetic nerve guide implants in humans: a comprehensive survey. Neurosurgery 59 (4), 740–747; discussion 747–748.

Neurotrophic factors

After implantation, the lumen of nerve guides shows elevated levels of cytokines and trophic factors. Cytokines are a broad family of small proteins secreted by cells that have a specific effect on cell–cell interactions and communication (see Chapter 4 **Cellular signaling**). An important neuropoietic cytokine released by glial cells as a response to injury is ciliary neurotrophic factor (CNTF) because it promotes cell survival or differentiation and can influence regenerative capacity. Other neurotrophic factors also play an important role in assisting neuron survival after severing of the axon (also called **axotomy**). These factors include neurotrophins and fibroblast growth factors and originate from Schwann cells in both the proximal and distal stumps of the severed nerve.[9] The neurotrophins include nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and brain-derived neurotrophic factor (BDNF). These molecules signal through tyrosine kinase (Trk) receptors in the cell membrane in conjunction with low-affinity receptors such as the low affinity nerve growth factor receptor (NGFr/p75). After injury, NGF is elevated in the distal nerve stump and acts through TrkA on sprouting nerve fibers to assist in the survival and outgrowth of a proportion of sensory neurons. Other sensory neurons express different receptors and are supported by NT-3[18] or by other factors such as glial-derived neurotrophic factor (GDNF). BDNF and NT-3, respectively, signal through TrkB and TrkC receptors that are present on alpha motor axons and increase neuron viability and sprouting after axotomy. Increased brainstem and spinal cord alpha-motor neuron survival has been reported using exogenously supplied GDNF and BDNF.[22] Nonviral neuronal targeted delivery of genes encoding for BDNF has also proven to be efficient in promoting neuroprotection and regeneration after an injury.[26] An in vivo study combining GDNF and NGF in delayed release profiles from nerve guides demonstrated a synergistic effect and significantly increased axonal regeneration above either GF alone or controls.[18,22]

There are several approaches to deliver neurotrophic factors in biocompatible nerve guides. The growth factor can be embedded within the nerve guide, homogeneously, or specifically bound to the luminal wall, effectively directing the release into the lumen.[26] The matrix in the lumen of the nerve guide is also an excellent substrate for binding growth factors; either simply adsorbed to the substrate surface, or as part of a noncovalent controlled release system. Heparin-binding growth factors (e.g., NGF, NT-3) can also be locally bound to the matrices allowing progressive delivery to regenerating axons.[23] The potential permutations and concentrations of neurotrophic factors are many; cocktails of different neurotrophic factors may result in enhanced neuron survival and gradients of such factors lead to directed axonal or cell growth (see Chapter 12 **Controlled release strategies in tissue engineering**). However, it may be more physiologically relevant to establish defined gradients of growth factors along the damaged nerve to prevent the potential "candy store" effect of axonal growth slowing or even stopping at the site of elevated factor delivery.

17.3.7 Biofabricated nerve guides

Biofabrication describes the use of 3D printing or other digital-based manufacturing approaches within tissue engineering (see Chapter 12 **Controlled release strategies in tissue engineering**). There are many different types of 3D printing technologies and many have been used to treat PNI.[27] The logical approach is to fabricate nerve guides with thin walls or in a specialized purpose. Injuries at the bifurcation (splitting) of the PN create challenges in standard nerve guide repair (Fig. 17.4). In

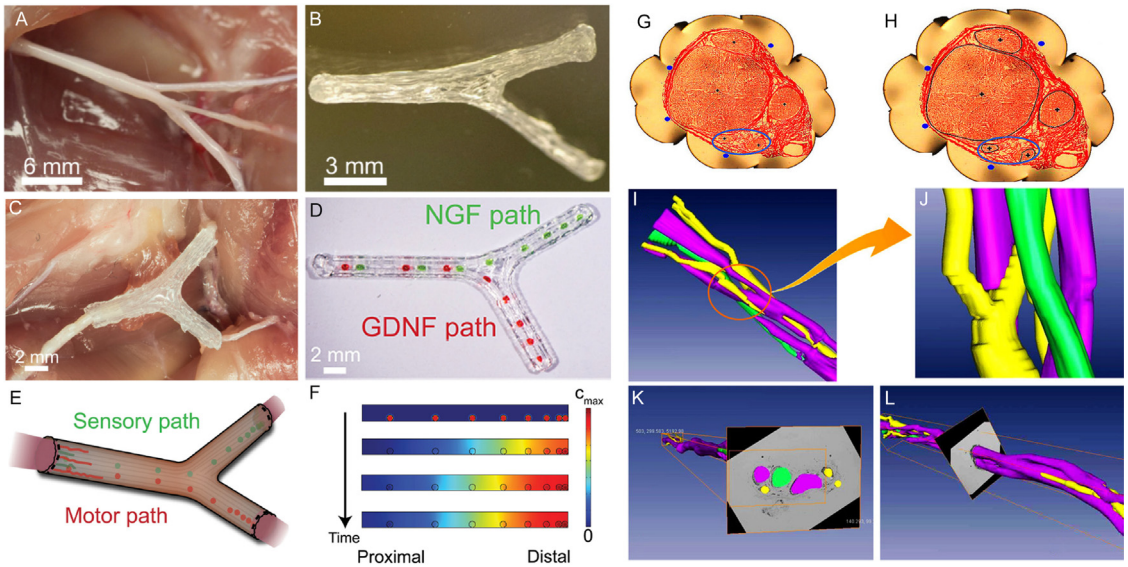

FIGURE 17.4

Examples of 3D printing and 3D rendering software for nerve guides. 3D printed construct (a—f) that allows splitting of the nerve guide. (g and h) Histological sections of human nerve allow the recreation of the tortuous 3D fascicules shown in (i—l). *(a—f) From Johnson et al., 2015. 3D printed anatomical nerve regeneration pathways. Adv Funct Mater 25 (39), 6205—6217. (g—l) From Zhong et al., 2015. Three-dimensional reconstruction of peripheral nerve internal fascicular groups. Sci Rep 5, 17168.*

this example, the bifurcated nerve was scanned, and a digital model developed that could be 3D printed using extrusion processing.[28] Furthermore, NGF and GDNF gradients were approximated using precise placement of matrices loaded with the appropriate growth factor (Fig. 17.4d—f). Fig. 17.4a—f highlights both the promise of biofabrication and challenges currently faced, with improvements in resolution levels still requiring the production of thin-walled structures with minimal profile differences such as shown in Fig. 17.4c.

Currently, the resolution of digital models and the ability to rapidly process data into customizable nerve guides[29] outpaces the ability to print the required resolutions. A common feature of fabricated nerve guides with internal structures is that there is low porosity within the nerve guide. For example, Fig. 17.4g—l are digital reconstructions of human nerve fascicles, reconstructed from histological sections. The tortuosity and thin structures that compose these guiding fascicles highlight how simple it is for a nonguided axon to grow into the incorrect fascicle at the distal end. Other imaging approaches also can identify fascicles, although these structures are too fine to be accurately printed.[30] With further improvements in 3D printing resolution, such fascicular structures could be accurately fabricated within nerve guides to increase the critical gap length.

17.3.8 Bioprinting for the PNS

Incorporating viable cells within biomaterials throughout the printing process with a technique known as **bioprinting** can be used to build cell-laden scaffolds that aim to mimic the structure and function of

neural tissue. Human gingiva–derived mesenchymal stem cells (MSCs) were 3D bioprinted into nerve constructs and have been shown to bridge segmental defects in rat facial nerves with successful functional recovery in vivo.[31] Gingiva-derived MSCs were chosen not only due to their capacity to self-renew and differentiate into functional glial and neuronal cells (feature common to all MSCs) but also due to their potential to be induced to neural progenitor-like cells (NPCs) with improved therapeutic effects on PN regeneration. Successful bioprinting of rat Schwann cells by extruding cell-laden fibrinogen into a thrombin solution was the first example of combining biomaterials to bioprint neural cells.[32] Other approaches to bioprint these cells have also been explored for various applications in the CNS (see Sections 17.3.7 and 17.4.4).

17.3.9 PNS summary

Small defects to the PN may be repaired by suturing nerve stumps together, but larger nerve gaps require additional approaches in order to bridge proximal and distal ends. The standard approach to connect the nerve stumps is autograft implantation, which endorses axon regrowth; however, besides causing a lesion at the donor site, it is rare that axon regrowth results in full reinnervation and return of sensory and motor functions to an injured patient. Alternatively, a biomaterials-based approach involves in vivo implantation of artificial scaffolds and substrates that will bridge the defect and guide axons with innate ability to regenerate. Current tissue engineering strategies to treat PNI with nerve guides range from Schwann cell or stem cell grafts, bundled fibers or channels, or oriented scaffolds/matrices placed within nerve guides. These approaches have been further paired with gene therapy or biochemically stimulated hydrogel filling in order to create a gradient that further promotes axonal regeneration following PNI.

17.4 CNS: spinal cord

Numerous animal trials to treat **SCI** have achieved some success although such approaches have yet to be successfully translated to the clinic. Damage to the spinal cord results in the immediate death of neural cells and disrupts the blood supply to the injury area. This in turn compromises the neuronal cell bodies present in the spinal cord, which presents a major difference to damage of the PNS, where only axons are initially affected. Furthermore, a **spinal injury scar** develops that impedes potential effective endogenous or treatment-driven regeneration. This scar consists mainly of reactive astrocytes, fibroblasts, pericytes, ECM, and myelin debris. These scar cells can express axonal growth inhibitory molecules such as chondroitin sulfate proteoglycans and semaphorins and so form a chemical and physical barrier for axon regeneration.[9,33] There are also immune/inflammatory changes and extensive neuronal and glial degeneration as a secondary response to the initial injury that further reduces the prospect for nervous tissue repair and functional recovery.

17.4.1 Summary of anatomy and injury response

The core of the spinal cord consists of a butterfly-shaped gray matter containing neuronal cells, support cells (termed **glia**), and numerous blood vessels (Fig. 17.5a). The white matter surrounding this structure contains mainly axons and **oligodendrocytes** (a type of glial cell that provides myelin insulation to CNS axons), astrocytes, microglia (immune cells), and relatively fewer blood vessels. The spinal cord is wrapped within multimembrane layers (dura mater, arachnoid, and pia matter) (Fig. 17.5b) that form the

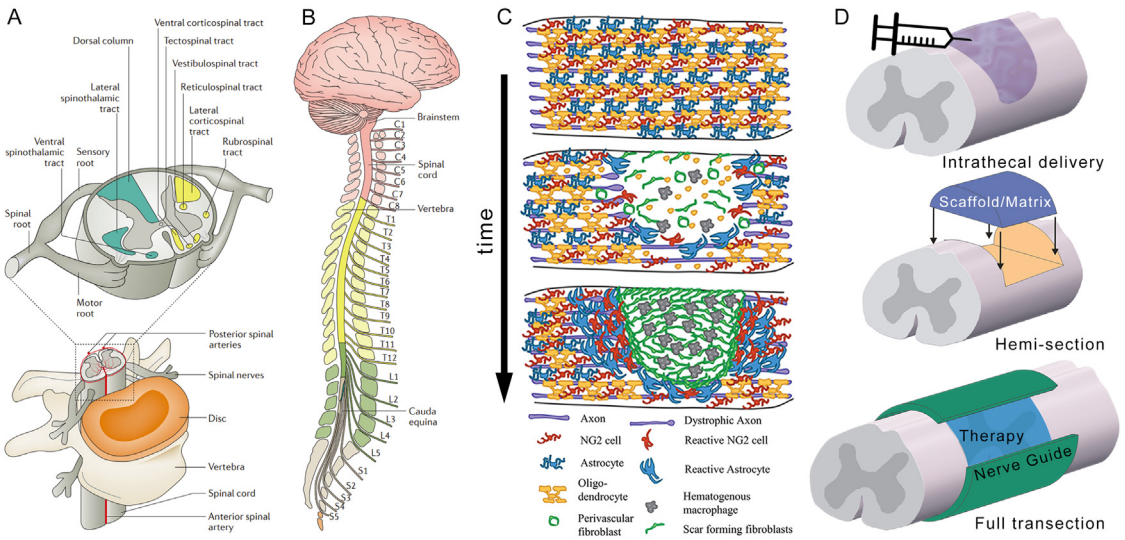

FIGURE 17.5

(a) Anatomy of the spinal cord including the surrounding anatomy of the spinal meninges (b), (c) schematic of a spinal cord scar formation, (d) intrathecal, hemisection, and full transection models for researching SCI models. *(a and b) From Ahuja et al., 2017. Traumatic spinal cord injury. (c) From Hackett and Lee, 2016. Understanding the NG2 glial scar after spinal cord injury. Front Neurol 7(199).*

intrathecal space in which the cerebrospinal fluid (CSF) circulates. SCI results in disruption of both sensory and motor axons, and this lack of anatomical and functional continuity in the spinal cord has devastating consequences for injured patients. The initial injury response following bruising or transection of the spinal cord involves a primary event in which cells and tissue are crushed or cut by the injury and cells undergo **necrosis**. The primary injury initiates secondary events that include altered local blood flow, activation of immune cells and influx of macrophages, ionic imbalance including raised Ca^{2+} levels, release of inflammatory cytokines, oxidative stress, and fluid build-up within the lesion site. Macrophage/microglia-mediated removal of damaged nervous tissue results in the formation of fluid-filled cysts (Fig. 17.5c). Secondary inflammatory events cause neurons in the gray matter, and oligodendrocytes in the white matter, to undergo **apoptosis**. Death of oligodendrocytes and subsequent loss of the myelin sheath interferes with the speed and reliability of electrical conduction in any remaining intact axons, significantly contributing to a sustained and long-lasting loss of function. Successful spinal cord repair requires all these issues to be targeted. This has proved to be a complex and difficult task.

The tissue engineering challenge to overcome spinal cord scarring

CNS white matter, which contains a range of myelin and myelin-associated axonal growth inhibitors, restricts any substantial axonal regenerative response in the adult spinal cord, regardless of injury. In a seminal paper by David and Aguayo in 1981, the transplantation of peripheral nerves into the spinal cord showed that it was the physical/molecular environment that prevented regeneration and not necessarily a factor that is intrinsic to CNS neurons.[34] The use of scaffolds and matrices in the spinal cord provides an extracellular environment for regrowth and repair-supporting molecules and/or cells

and/or provides a terrain for severed ascending (sensory) or descending (motor) axons to grow across the site of injury. Acellular substrates (scaffolds/matrices) may elicit an axonal regeneration response[35] but typically are less effective than cellular transplants.

After SCI, a physical and chemical barrier (fibro-adhesive and glial scar) forms at the impact site which protects adjacent, spared CNS tissues from further damage by infiltrating monocytes but also limits the growth of new axonal sprouts. This scar consists mainly of reactive astrocytes, subtypes of pericytes and meningeal fibroblasts, which express extracellular axonal growth inhibitory molecules such as chondroitin sulfate proteoglycans and semaphorins. It has become clear that manipulation of this barrier will be an essential component of a successful repair strategy for the injured spinal cord. Such manipulation of the scar may include the following: (1) decreasing the inhibitory nature of the scar, (2) preventing axons from recognizing inhibitory molecules, and (3) enhancing the intrinsic growth ability of axons. The permissiveness of the scar for growing axons can be increased by blocking receptor–ligand binding. This reduces the synthesis of inhibitors or degrades biologically active components of the inhibitors, for instance, using the chondroitinase enzyme. By reducing the expression of growth inhibitors, cells can to some extent recover their ability to form and extend nerve processes, including axons. Examples of current approaches to promote regrowth include cell therapies and introducing inhibition antagonists,[36] the latter already the subject of clinical trials.[37]

In the laboratory, several experimental approaches resulted in axonal regrowth through and beyond the scar, but success was usually obtained using injuries that could be considered as "smaller size" injuries, i.e., with less scar tissue formation. The barrier of scarring can be overcome by implantation of olfactory ensheathing glia (OEG) into the scar-forming environment,[38,39] increasing the levels of neurotrophic factors in the nervous tissue using osmotic mini-pumps[40,41] or the use of a modified form of the chondroitinase enzyme.[42,43] Understanding mechanisms of reduced cyst formation and astrocytic-fibro-adhesive scarring associated with tissue engineering scaffolds and matrices is a fundamental area of investigation.[44–46] Combining this knowledge is essential to develop scaffold- and matrix-based regenerative strategies that aim to bridge the lesion that results from SCI.

17.4.2 SCI models

There are numerous experimental animal models of SCI, mostly involving rodents (Fig. 17.5d). Different SCI models are best suited for the measurement of tissue sparing (contusion/compression), axonal regeneration (complete transection), or sprouting of intact fibers (hemisection). When using these models, it should always be borne in mind that the human spinal cord is comparatively very much greater in length and with some key differences in intrinsic organization,[47] and with chronic responses to injury that impede sensorimotor recovery.[48,49]

Contusion animal model

Most human SCI is the result of a compression or **contusion injury**, and usually leads to the formation of cystic cavities as schematically shown in Fig. 17.5c. Experimentally, such injuries are modeled either by transient compression of the spinal cord using a weight drop method or a compression clip, or by displacement of the spinal cord using an inflatable device (e.g., the Fogarty inflatable balloon). With contusion/compression injuries, analysis of the axonal regeneration response is complex due to the

presence of spared axons and their collateral sprouts. To repair the contused/compressed spinal cord, cells or materials can be injected directly into, or next to, the contusion cavity, in small volumes. These injections into the lesion cavity may likely elicit a neuroprotective effect and/or an axonal regeneration response which could support some degree of functional recovery.

Hemisection model

Some instances of human SCI result from a laceration with disruption of the dura mater. This injury is mimicked in the laboratory using a surgical microknife or microscissors as depicted in **17.5D**. Although with a partial (hemi) section the loss of tissue and function is limited, over time injury-induced secondary tissue loss may add to the overall injury zone. With a **hemisection SCI model**, as with a contusion/compression injury, the presence of spared axons that may form axon collaterals makes a definitive analysis of any transplant-induced regeneration response difficult. To repair the spinal cord following a hemisection, a bridging transplant can be placed in the lesion site. Examples of bridging transplants include preformed scaffolds with or without cell suspensions/in situ hydrogels[42] and PN segments.[41] While the grafted cells/tissues/scaffolds may elicit axonal growth in and across the hemisection, responding axons usually fail to exit the transplant without an additional intervention that facilitates their growth across the transplant–spinal cord interface. Examples of such additional interventions are increasing neurotrophic levels using osmotic minipumps or direct intraparenchymal injections (see Section 17.9) or introducing modified chondroitinase.[42]

Full transection models

Full transection experiments (Fig. 17.5d) are not common due to surgical complications such as instability of the spinal column and the difficulty in animal care. However, this type of lesion, while less clinically relevant, can provide absolute evidence of true axonal regeneration. While hollow poly(acrylonitrile-co-vinyl chloride) nerve guides have been used for full transection (see Section 17.9), other investigations have used soft, flexible hydrogel nerve guides in the fully transected cord and demonstrate improved regenerative capacity.[50,51]

Intrathecal delivery

The many blood vessels and capillaries that pass through the CNS (the gray matter is particularly highly vascularized) have ordered endothelial cells, tight junction molecules, and astrocytic end-feet that between them form the **blood–brain barrier** (BBB) and blood–spinal cord barrier. These blood–CNS barriers ensure that the two environments can be separated and many proteins and other molecules are not able to freely pass across them unless facilitated by, for example, nanodelivery systems.[52] One way to deliver drugs to the CNS without having to cross the BBB is by lumbar puncture. An alternative way to deliver drugs to the brain is to inject fast-gelling adhering matrices with the drug into the space between the arachnoid and pia mater (intrathecal space) where the CSF flows.

A thermo-reversible hydrogel matrix blend of hyaluronic acid and methyl cellulose, called HAMC, was injected in the intrathecal space to successfully deliver modulatory drugs. The injectable and intrathecal delivery approach has flexibility in that cells or drugs can be delivered to modulate inflammation and limit the extent of injury.[44]

Gender and age

Gender differences may affect the cascade of degenerative processes and functional outcomes after traumatic and ischemic CNS injuries, although there is some controversy.[56] While some studies argue that the presence of estrogens and progesterone likely plays an important role in the greater neuroprotection sometimes reported in females versus males after injury, the underlying biological mechanisms are not fully understood. Alternatively, other studies question the involvement of female sex hormones in outcome in specific SCI models and claim absence of gender differences. The importance of age in the pathophysiology of CNS and PNS injuries and in the ability to recover is well established. However, animal models do not always reflect these gender and age differences, and it may well be that for a particular type of injury different animal models should be developed that represent such differences.

Species selection for SCI models

The selection of the species for SCI studies is an important issue. Tissue engineering strategies have focused on the mammalian model, while invertebrates and nonmammalian vertebrates are aimed at understanding fundamental biochemical and molecular processes associated with cell survival and regeneration. The pathology of rat SCI is fairly similar to that in humans, although important differences have been discerned, including reduced glial scarring, inflammation and demyelination, increased Schwann cell infiltration, and protracted Wallerian degeneration in humans.[49] Mice are also commonly used and have the advantage that numerous genetically engineered strains are available. A notable difference between mouse and human SCI is that cysts usually do not form in mice. Note also that there are important strain-specific differences in how animals respond to experimental spinal cord trauma.

Larger species such as pigs, dogs, cats, and nonhuman primates are also used in SCI studies, with the latter being most relevant to the human condition. To assess the efficacy of specific approaches to repair the human spinal cord, it is increasingly accepted that the development and use of nonhuman primate models such as monkeys is advisable. However, in addition to ethical issues, the maintenance of the injured primate is extremely labor intensive. Alternatively, cats can also be used to model SCI because they offer important advantages over other models in that their spinal cord physiology is well described, and these animals can be trained relatively easily. Treadmill training of cats allows the animal to regain their ability to bear weight after spinal injury, whereas untrained animals show limited locomotor recovery. This difference in behavior when comparing untrained versus trained cats becomes a disadvantage to this animal model for SCI because experimental results are not representative of the proposed therapy. Locomotion recovery in cats after injury is strongly influenced by their **central pattern generator** in the spinal cord, enabling recovery of reflex stepping on a treadmill. The pattern generator in humans appears to be comparatively less effective after SCI.

17.4.3 Cell transplantation

Cell transplants into the spinal cord aim to encourage injured axons to grow through areas of damage toward and into their target areas, and/or as a means to deliver repair-supporting molecules. Various cell types have been tested including Schwann cells, OEG, and stem cells derived from either embryonic or adult tissues.

Schwann cells

Schwann cells can produce a variety of growth-promoting molecules that provide a growth substrate for injured CNS axons. In the injured spinal cord, Schwann cell transplants elicit axonal regeneration (see Section 17.9).[38,54,57] Schwann cells also form myelin sheaths around CNS axons allowing signal conduction in regenerated CNS axons. In addition to axonal regeneration, an intraspinal Schwann cell graft promotes neuroprotection thereby reducing the additional loss of nervous tissue due to secondary events.[38] Schwann cells grafted into the lesioned CNS show little or no tendency to migrate and integrate with the host tissues due to a mutual repulsion exhibited between Schwann cells and CNS-derived astrocytes, as demonstrated in vitro.[33] Schwann cell transplants in the cystic cavity combined with maintaining an increased level of cyclic adenosine monophosphate result in improved functional recovery.[58]

Olfactory ensheathing glia

The environment of the adult mammalian brain and spinal cord is generally nonpermissive for regrowth; however, there is one structure in the mature CNS where axonal growth does occur: the olfactory bulb. Throughout adulthood, newly formed olfactory neurons in the upper nasal cavity generate axons that grow through the cribriform plate into the olfactory bulb to make their appropriate connections in the brain. This particular growth response occurs because of the presence of OEG, which helps to shield the growing axons from nonpermissive CNS tissue. When grafted into the injured spinal cord, OEG can promote the regrowth of axons beyond areas of injury and into distal spinal tissue (see Section 17.9). The underlying mechanisms are still unclear, but it is possible that OEG demonstrate substantial migration in the host CNS and prevent axons from recognizing the nonpermissive adult CNS environment by ensheathing them, as they normally do for growing olfactory axons. In addition, OEG may support axonal growth by eliciting only a moderate astrocytic reaction.

Stem cells

The application of stem cells for spinal cord repair has to date been limited by technical and ethical concerns and, sometimes, political restrictions. However, different types of stem cells, such as embryonic or adult neural stem cells[59,60] or stem cell-like bone marrow stromal cells,[47,61] were shown to elicit neuroprotective and/or regenerative responses. Induced pluripotent stem cells (iPSCs) have also been trialed.[62] The mechanisms underlying the trophic effects of stem cell transplants are outlined in Chapter 2 **Stem cells**. The secretion of factors that support the repair of existing blood vessels and/or formation of new blood vessels is considered an important determinant in stem cell—based nervous system repair.[63] It may be necessary to differentiate stem cells before/during cell transplantation, as the injury environment does not normally favor differentiation into neurons or oligodendrocytes. Differentiation of stem cells prior to implantation is accomplished by a variety of factors (see Section 17.4.2), although the most efficient combination has yet to be determined experimentally. In vitro differentiation of stem cells or glial restricted precursors to astrocytes via pretreatment with either BMP4 or CNTF results in populations of cells with different properties, which consequently exert different effects on tissue repair when injected into animals with experimental SCI. Astrocytes derived by BMP4 reduce scarring at the lesion site and support axonal regeneration, whereas astrocytes derived by CNTF treatment promote scarring, reduce axonal regeneration, and even induce extreme—often painful—sensitivity to normal stimuli (termed allodynia).[64,65] It seems clear that not all stem cell or progenitor-derived cells are

beneficial to tissue repair and functional recovery. It has been reported that even intravenously injected stem cells do not need to reach the injured spinal cord to have a beneficial effect. The administered cells are thought to act as decoys to reduce host immune attack on the injured spinal tissue, thus reducing secondary injury effects.[66] In all instances, it is essential to ensure that stem cells do not continue to multiply after transplantation, which could potentially lead to the formation of tumor-like structures.

Genetically modified cells

Gene therapy holds great potential for the treatment of neurotrauma and many types of neurodegenerative CNS disease. Delivery of therapeutic molecules by gene therapy may support the arrest or delay of secondary neurodegeneration and stimulate the regenerative capacity of CNS neurons. This involves using different cell types including Schwann cells, fibroblasts, OEG, and neural progenitors.

Gene therapy allows selective delivery of neurotrophic factors to specific CNS locations. For example, OEG genetically modified to secrete neurotrophins such as NT-3, BDNF, and GDNF have stimulated long distance growth in certain spinal cord tracts.[67] In addition, neurotrophin-secreting OEG improve spinal tissue sparing, which may result in improved behavioral outcomes.[38] Genetic engineering of cells can involve the use of different viral vector systems and these require hurdles to be overcome for clinical applications. The transfection of donor cells enables better monitoring of the cells within the spinal cord or CNS when using reporter genes. It is also possible to use gene therapy to drive astrocyte-to-neuron conversion in the injured spinal cord using a viral vector to overexpress the transcription factor NeuroD1.[68]

17.4.4 Nanomedicine to treat SCI

Because surgery of the spinal cord often involves substantial risk, relatively **minimal invasive** approaches such as systemic or intrathecal injection are preferred. Nanoparticles (NPs) represent one injectable option and have a high permeability while containing hydrophobic drugs that can be released in a time-dependent manner. Regardless of classification—inorganic, lipidic, polymeric, and dendritic—NPs are able to bind/interact to drugs via functional groups. As portrayed in Fig. 17.6, NPs have tuneable properties such as surface charge (positive, negative, or neutral) and can be functionalized with different targeting ligands, such as aptamers, antibodies, peptides, and proteins.[69]

One example of this is the suspension of methylprednisolone-loaded PLGA NPs in agarose that is administered locally after a spinal cord hemisection.[70] The delivery of loaded NPs in agarose resulted in improved locomotion, reduced scarring, and decreased inflammation, relative to systemic injection, or local delivery of agarose loaded with blank NPs.

Using NPs targeted at macrophages is another approach to reduce the secondary degenerative event and address neuroinflammation, which is one of the most relevant mechanisms involved in the progression of secondary damage. Polymeric NPs can be used to selectively target macrophages/monocytes due to their specific endocytic/phagocytic activity, because it is known that these cells are recruited to the primary injury zone. Injecting NPs to alter macrophage polarization can reduce the levels of scarring after SCI.[71] NPs offer great potential to target other tissues of the CNS such as the brain (see Section 17.4).

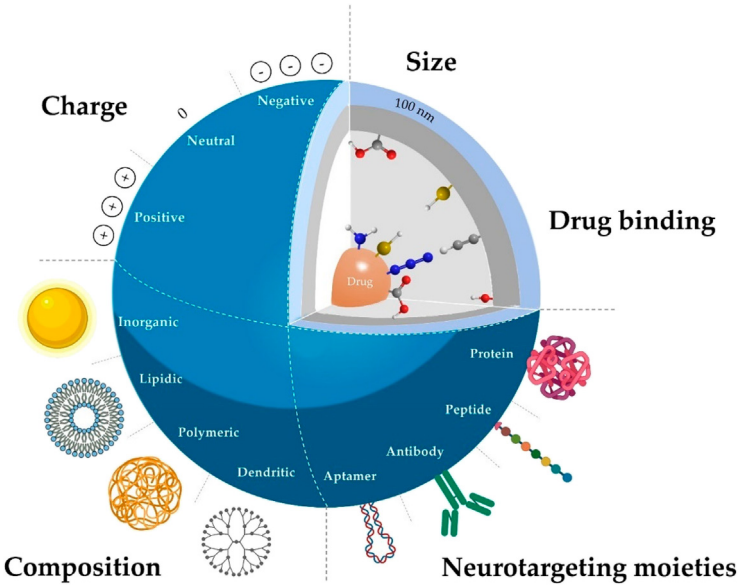

FIGURE 17.6

Main features of the nanoparticles influencing delivery efficiency. *From Spencer et al., 2020. Breaking barriers: bioinspired strategies for targeted neuronal delivery to the central nervous system. Pharmaceutics 12 (2).*

17.4.5 Matrices and scaffolds

An implanted matrix/substrate must allow regenerating axons to penetrate, grow through, and then exit the implant so that the axons can reenter and reestablish connections with appropriate target regions. After SCI, both descending and ascending axonal tracts are disrupted, each of these tracts having specific locations in spinal cord white matter. Since various axonal populations have different growth requirements, a future graft may consist of composite bridging materials, each tailored to a particular axonal population. Whether matrices or scaffolds are effective due to regenerative properties, or as a result of other factors such as neuroprotection, is a matter still under investigation.[72]

Many biomaterials, either natural (e.g., collagen, alginate, agarose, chitosan, fibrin, fibronectin, Matrigel) or synthetic (e.g., methyl cellulose, nitrocellulose, poly(ethylene glycol) (PEG), nanofibers), encourage some degree of tissue preservation and perhaps fiber tract repair after SCI.[35] The implantation of such scaffolds into the injured spinal cord often results in a significantly different injury response to that commonly observed with a traumatic lesion alone. Cystic cavity formation can be completely absent when scaffolds are implanted, and the density of astroglia scarring is often reduced. However, some materials such as collagen Type 1 (in the absence of accompanying donor cells or functional molecules) may induce a detrimental encapsulating response from fibroblast-like cells that interferes with implant-host integration. When such scarring can be reduced or avoided by the use of other materials, scaffolds engineered with oriented frameworks or pores are capable of supporting axonal ingrowth in a highly directional and ordered manner.[35]

Relevant peptide sequences

For many scaffolds and matrices, there is often a need to direct and target cell/material interactions more specifically than full protein inclusion or adsorption. The conjugation of bioactive peptides with a material is an effective way to influence cell/material interactions, although this has largely been demonstrated only in in vitro environments. Many peptides reproduce various bioactive domains of laminin and include YIGSR, IKVAV, RGDS, and RNIAEIIKDI (Arg-Asn-Ile-Ala-Glu-Ile-Ile-Lys-Asp-Ile).[9] The peptide sequence YIGSR is often preferred as a peptide ligand for in vitro applications and is an alternative to RGD for cell adhesion (see Chapter 5 **Extracellular matrix as a bioscaffold for tissue engineering**).

The IKVAV sequence promotes cell adhesion, neurite outgrowth, experimental metastasis, collagenase IV activity, angiogenesis, plasminogen activator activation, cell growth, tumor growth, and differentiation of NPCs.[9] Although the aforementioned active peptide sequences are effective at promoting neuron adhesion and axonal growth alone, the use of both YIGSR and IKVAV has a synergistic effect on neurite outgrowth. The extended peptides CDPGYIGSR and/or CQAASIKVAV further increase potency[73] over the shortened versions but are more expensive to synthesize.

In addition to the conjugation of bioactive peptides with a material, another mechanism of presenting these bioactive sequences is the use of peptide-based materials, such as self-assembling peptides (SAPs). Here, the peptides themselves form the backbone of the material, leading to the high-density presentation of the desired peptide epitope without the need for any additional conjugation steps. This has been demonstrated, for example, by the fabrication of hydrogels containing one or more fluorenylmethoxycarbonyl-(Fmoc-) SAPs, including IKVAV- and RGD-based SAPs, that support neural cell growth in vitro and in vivo.[44,74]

Matrices

The number of in situ gelling matrices to treat SCI is ever increasing. There are strong arguments for combining cell therapies with injectable matrices to protect cells from membrane shearing and death, similar to issues found in 3D bioprinting. In situ gelling hydrogels can be classed as chemically or physically cross-linked, with the latter having a broader range of materials. The types of gelation and reported hydrogels combined with cells and/or biologically active factors used to improve SC regeneration are shown in Fig. 17.7.

In situ gelling matrices are important to maintain a minimally invasive approach to treating SCI. Hyaluronan-Methyl cellulose (HAMC) is being developed as a flexible injectable technology for importing both drugs and cells into the lesioned CNS. Additionally, exploiting the properties of charged SAPs within these gelling matrices offers a platform that can encapsulate neurons and other cells for SCI. Aggregation of the SAP solution after mixing the peptide solution with cell suspensions has been used in numerous SCI studies that demonstrated reduced cavitation and some regeneration.[75,76] Fibrin is also commonly used as a matrix that can be modified with peptide sequences or used to deliver neurotrophic factors with a heparin binding site.[23,77] Fibrin also resulted in the greatest number of neurons traced with retrograde labeling in full transection studies, compared to collagen Type I, methyl cellulose, and Matrigel.[50] Matrigel has also been used in many CNS tissue engineering investigations, particularly with Schwann cell or OEG transplantation into the spinal cord (see Section 17.9). In many of the above-mentioned matrices injected into the spinal cord, reduced scarring is a beneficial outcome.

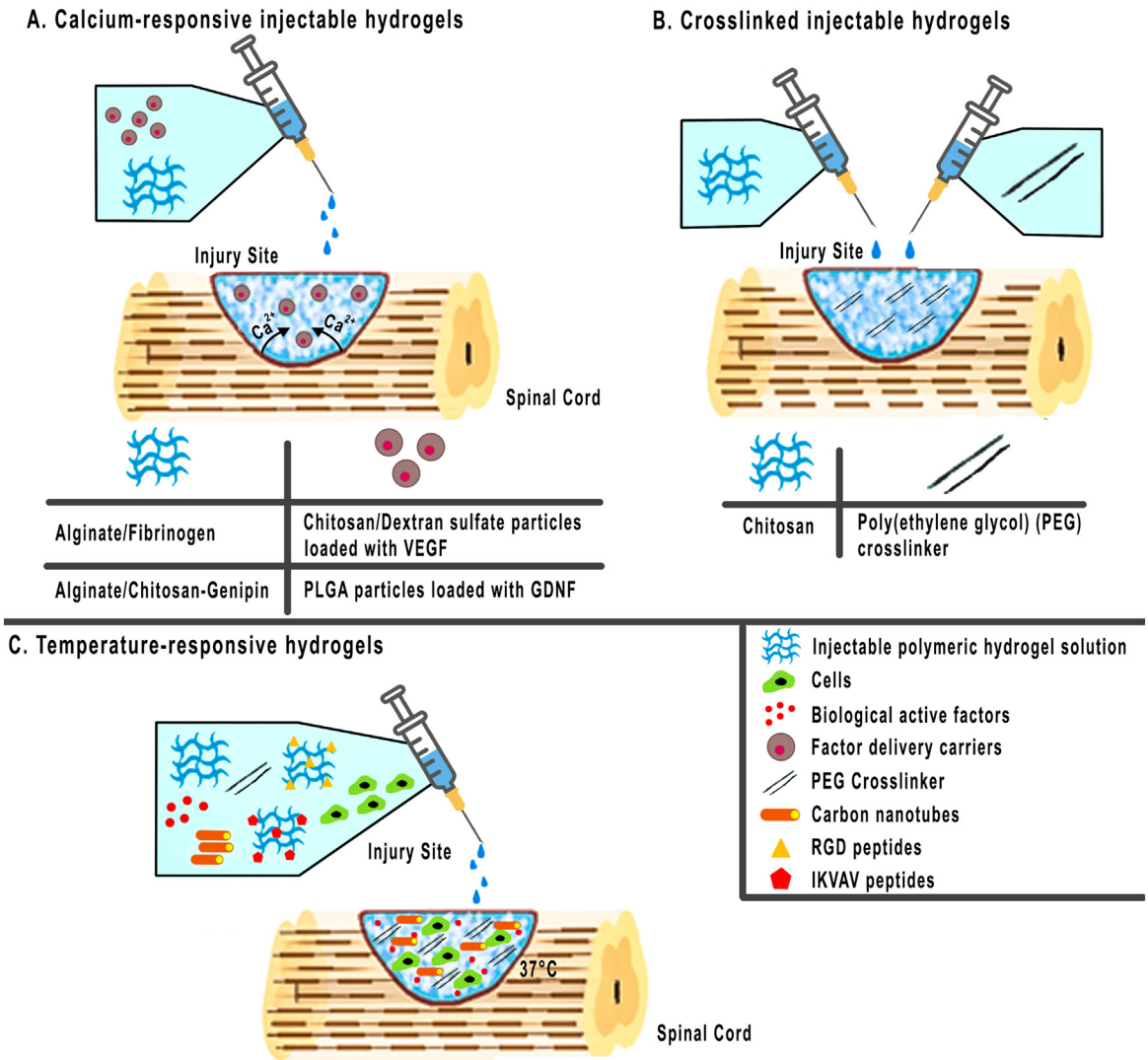

FIGURE 17.7

In situ injectable hydrogels. (a—c) Types of gelation. *From Morgado et al., 2019. In situ injectable hydrogels for spinal cord regeneration: advances from the last 10 years. Biomed Phys Eng Exp 6 (1).*

Scaffolds

Scaffolds made from many different materials have been implanted into the spinal cord.[35] Porous scaffolds can be a valuable platform for cell delivery and a tool to elicit neuronal differentiation and functional integration of the delivered cells in the damaged spinal cord.[78] Alternatively, decellularized tissue has been investigated; however, this is currently limited in scope. Oriented scaffolds, particularly hydrogel scaffolds, have demonstrated their ability to stimulate axonal growth. Similar to scaffolds used for PNI, controlled uniaxial freezing can generate channels within a polymer solution or hydrogels by using the forces associated with crystal growth. The resulting oriented scaffolds demonstrate neurite and cell penetration into the channels.[12]

17.4.6 Nerve guides in the CNS

The use of cell transplantation with nerve guides in the fully transected spinal cord was pioneered by the Bunge group (see Section 17.9) with poly(acrylonitrile-co-vinyl chloride) (P(AN-VC)) nerve guides. Collagen or pHEMA nerve guides can also be used to promote regeneration in the spinal cord.[50] Regeneration into P(AN-VC) nerve guides occurs with both Matrigel and Schwann cells present in the lumen. As is the case with PN repair, the inclusion of a matrix into a hydrogel nerve guide increases the number of axons.[50]

17.4.7 Summary

SCI initiates a plethora of destructive events. Some of the cells and molecules present at the site of injury may contribute to protection and repair, but the ultimate outcome is loss of nervous tissue and loss of motor, sensory, and autonomic function. Unlike the PNS and the use of PN autografts and nerve bridges, there is currently no reproducible or widely accepted clinical therapy for SCI. One of the main obstacles for regenerating axons in the injured spinal cord is the presence of a spinal injury scar. Repair is also compromised by host immune and inflammatory responses and associated secondary injury cascades that exacerbate any primary injury effects. Therapies that reduce these adverse events will have beneficial effects on spinal cord repair. Cell transplantation combined with supportive matrices and scaffolds or neurotrophic factors has been the predominant focus of tissue engineering strategies. The emphasis has been on neuroprotection, to elicit nervous enhanced tissue sparing, and reestablishment of continuity across an injury site, to reinstate axonal circuits involved in function. Future objectives include increasing the number of damaged ascending and descending axons that regenerate, improving the remyelination of these regenerating axons, the guiding of regenerating axons back to their appropriate target regions in the brain or spinal cord. The development of appropriate animal models for SCI has been a focus of research, with recent endeavors translating to the field of microfluidics that take advantage of small-scale in vitro systems that aim to mimic the disease model. Furthermore, the development of new techniques involving gene therapy and accurate cell dispensing prior to transplantation provides additional approaches to treat this injury. Also critical is where, when, and for how long to apply particular factors.

17.5 CNS: brain

17.5.1 Trauma and stroke

Traumatic lesions to the brain or blockage of blood supply to the brain (stroke) prevent oxygen and nutrient flow. In both clinical conditions, neuronal death occurs almost immediately, and urgent intervention prevents further deterioration. Reconstruction of gray and white matter defects in the brain is especially difficult after large lesions (e.g., stroke or closed head injury) or in the chronic, degenerative situation. In addition to neuronal death, there is demyelination, retraction, and/or aberrant sprouting of injured axons, glial/fibro-adhesive scarring, and there may be progressive tissue cavitation. Under these circumstances, there is a need for cell replacement and some form of neural tissue engineering to develop scaffolds that facilitate reconstruction and restore continuity across a traumatized region.

While some studies have investigated the deployment of biomaterials, usually hydrogels, to act as a biobridge from the adult stem cell niche to the injury site,[89] cell transplantation clinically has demonstrated the most promise.[90,91] In this regard, biomaterials that have the capacity to provide a safe sanctuary **microenvironment**,[92,93] while also attenuating inflammation have improved the efficacy of cell transplantation.[94] For instance, scaffolds have recently been used as biobridges, in combination

with human stem cell transplantation, within a stroke cavity generated in a rat model.[95] In this instance, SAP hydrogels were used due to their ability to present IKVAV, an epitope prevalent in laminin that is the major extracellular protein in the brain, in high density. The scaffolds supported the human stem cell graft integration, which was associated with continually improved motor function over the 9-month duration of the study, with coordinated paw movement recovering to a level not statistically separable to intact controls. When human stem cells were delivered in combination with the SAP scaffold, the grafts were larger; there was superior neuronal differentiation and subsequent integration, as demonstrated via electrophysiology data.[95]

17.5.2 Neurodegenerative diseases

Neurodegenerative diseases are often associated with substantial neuronal loss, the pattern of loss varying dependent on the type of disease process. Supply of appropriate neurotrophic factors via grafted donor cells or from slow-release polymers may protect compromised host neurons, but alternative cell replacement strategies may also be required.

Some of these neurodegenerative disorders, such as Huntington's disease (HD) and spinocerebellar ataxia, are inheritable and associated with single gene mutations. Other motor neuron disorders and diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) have more complex etiology, with only a percentage directly associated with dysfunction of a specific gene. It is likely that many of these degenerative conditions are affected by environmental factors as well as some form of genetic susceptibility. AD and PD in particular are diseases associated with the elderly and are becoming increasingly common as life expectancy increases and the population ages.

Early diagnosis and prevention of further deterioration would be the optimal ways of treating these neurodegenerative disorders. However, it is often the case that when symptoms become apparent there has already been substantial neuronal death. Engraftment of encapsulated cells that secrete the appropriate growth factor(s) has been trialed in experimental models of PD, AD, HD, amyotrophic lateral sclerosis (ALS) (loss of cortical motor neurons and spinal cord alpha-motor neurons), and chronic pain.[96]

Other tissue engineering strategies involve attempts to replace the lost neurons with new cells to be incorporated into host circuitries at either physiological or ectopic locations. PD is a multisystem disease, a major feature being the catastrophic loss of neurons in the midbrain that secrete dopamine. In numerous clinical trials, hundreds of PD patients have received intracerebral grafts of dopamine-rich fetal human brain tissue, many of these recipients experiencing some alleviation of their symptoms. Potentially alternative sources of tissue include xenografts and stem cells or NPCs modified to secrete dopamine. For example, human pluripotent stem cells have been ectopically transplanted into the brain of mice using a tissue-specific hydrogel. Importantly, this hydrogel was programmed to support these progenitor cells in two different ways. Firstly, they were rationally designed to be biocompatible with dopaminergic precursor cells and in fact biased the differentiation of grafted cells to see a 51% increase in a specific population of neurons lost in PD and critical for motor function. Secondly, controlled and sustained presentation of GDNF within the graft core was achieved, inducing a 2.7-fold increase in dopaminergic neurons and enhanced plasticity. Taken together, this resulted in significant restoration of motor function in parkinsonian animals over a 6-month duration.[97] Such approaches, perhaps combined with neuroprotective gene therapy, are potentially also of use in the treatment of motor neuron diseases and HD, where some recent studies have utilized biomaterials to improve the efficacy of viral vector gene therapy.[98]

17.5.3 Drug delivery to the brain

To reach specific areas of the brain, a non- or minimally invasive delivery of the therapy is key. The use of NPs as delivery systems for difficult-to-access parts of the body is one exciting area of research, while the imaging of tumors using NPs is another active area of research.[99] For many drug delivery systems to the CNS, the BBB is a key aspect to consider, confounded by the fact that many systemically injected NPs are rapidly removed from circulation. However, there are instances when the permeability of vasculature is significantly altered through injury or disease. One example is the period following injury or stroke, where there is a temporary increase in BBB permeability.[100] A cell surface receptor for self-recognition, CD47, has been recombinantly produced and attached onto NPs to significantly increase circulation times.[101] Despite the selective barrier function of the BBB, a number of papers have demonstrated that NPs can cross from the bloodstream into the brain, although there is evidence that serum protein binding to NPs (protein corona) can be problematic.[102]

Alternatively, NPs can be delivered to the CNS via intrathecal delivery[103] within an in situ gelling hydrogel to control their release. Intranasal delivery is also noninvasive route for delivering NPs to the brain and drug delivery in general.[104] More recently, studies have exploited the diffusion kinetics of drugs from biomaterials to achieve localized delivery of multiple drugs simultaneously, while importantly avoiding systemic delivery and the many problems associated with off-target effects. For instance, biomaterials have been deployed on the surface of the cortex to deliver both cyclosporin and erythropoietin simultaneously, with the drugs being shown to be localized and bioavailable for at least 32 days. This resulted in increased plasticity in the striatum and stimulation of endogenous NPCs in a relatively minimal invasive manner.[105] Such approaches, including localized and controlled gene therapy, are critical to the future of brain repair research to replicate embryogenesis and/or promote adult neurogenesis.

Brain targeted therapies

In the brain, successful delivery of drugs requires that they bypass the various barriers that separate the bloodstream from the brain **parenchyma**.[69] As described above, the BBB presents the major obstacle in systemic brain therapies due to the strong tight junction between endothelial cells, as portrayed in Fig. 17.8. This endothelial layer is regulated primarily by astrocytes and pericytes. To overcome the BBB, drugs may be directly delivered to the extracellular space with a catheter. This administration route takes advantage of the spaces between cells (interstitial pathway) to attain a homogeneous perfusion throughout the brain. This approach has been successfully employed with different types of delivery nanosystems, such as polymeric NPs. NPs may also be delivered to the brain via intraarterial injections, which take advantage of the major blood supplier to the brain, the carotid artery.

Overcoming the physical barriers that separate circulation between the body and the brain can further be paired with neural-targeted delivery. The successful delivery of drugs to neurons depends on the presence of exclusive receptors called neurospecific receptors. Other considerations are the competitive nature of other molecules, or ligands, that bind to the same receptor. Ligand–receptor interaction strength greatly influences the delivery rate, which is further influenced by the optimal ligand density, ligand mobility, and correct exposure of the targeting ligands. Choosing the correct ligand–receptor and tailoring the factors that affect drug delivery is crucial for developing effective therapies.

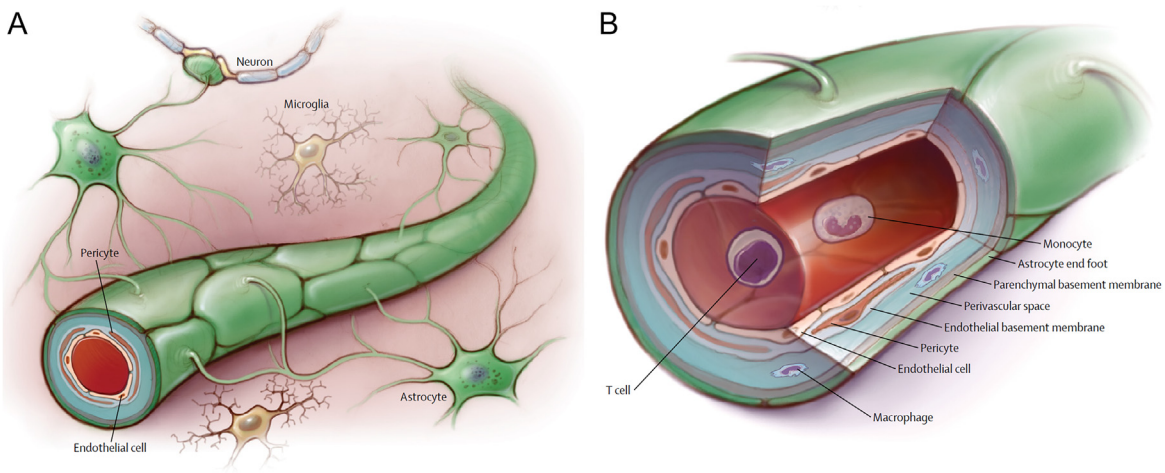

FIGURE 17.8

Schematic of the neurovascular unit. The blood—brain barrier separates CNS tissue from circulating blood and limits the effective intravenous delivery of many pharmaceutics. *From Pollak, T.A., et al., 2018. The blood brain barrier in psychosis. Lancet Psychiatr 5 (1), 79—92.*

17.5.4 Bioprinting for the brain

An early approach to incorporate neural cells within biomaterial-based inks to create bioprinted scaffolds demonstrated the possibility of 3D coculture of rat embryo neurons and astrocytes incorporated in collagen.[81] More recently, mouse primary cortical neurons were incorporated in a gellan gum bioink modified with RGD, a peptide sequence known to improve cell adhesion. As a result, 3D brain-like structures were successfully developed via extrusion-based bioprinting.[106]

Recent studies using a bioink that combined alginate, chitosan, and agarose have demonstrated successful in situ expansion and sequential differentiation of human iPSCs.[107] iPSCs derived from somatic cells are reprogrammed into a pluripotent state and can bypass issues associated with immune rejection and ethical concerns when compared to stem cells. Human iPSCs were successfully differentiated using the compound bioink described above, resulting in brain-like tissue with functional migrating neurons and neuroglia. This study presents perhaps a cornerstone that contributes to the potential of bioprinting in fabricating structures that aim to mimic functional neural networks in the brain.

17.6 CNS: optic nerve

Damage to fiber tracts interconnecting different CNS regions causes disruption of neural circuits and often leads to the death of associated neurons. As already mentioned for SCI, therapeutic strategies are therefore needed to ensure the viability of affected neurons, promote the regrowth of damaged axons across lesion sites, maintain growth within CNS tissue distal to the injury, and finally ensure that regrowing axons reinnervate appropriate target areas and reconnect with relevant target neurons.

The visual system is a useful model[108] in which to study regenerative success or failure after nerve fiber damage in the mature CNS since

(i) Retinal ganglion cells (RGCs), the neurons that project axons out of the retina through the optic nerve and via the optic chiasm and optic tracts to central targets in the brain, can be directly targeted via intraocular injections.
(ii) There is detailed knowledge of intraretinal and visual brain circuitry.
(iii) The optic nerve is a discrete, centrally derived tract that is surgically accessible and can be manipulated without affecting other fiber tracts and structures.
(iv) The extent of RGC survival and axonal regrowth can readily be quantified.

Normally, mature mammalian RGCs do not regrow axons beyond an optic nerve injury and, if the axotomy occurs within the orbit, about 90% of RGCs die within 2 weeks. This lack of regrowth is due to factors both intrinsic and extrinsic to the adult RGC.[109] However, some adult RGCs will survive axotomy and regrow their axons when appropriately stimulated and/or when provided with an appropriate local environment.

17.6.1 Regenerative therapies

After optic nerve crush, adult RGC survival can be enhanced by intraocular injections of recombinant neurotrophic factors such as BDNF, NT-4/5, CNTF, or GDNF or by injections of factors that block intracellular signaling pathways associated with cell death. However, these effects are transient, and viability is in most cases enhanced for only a few weeks. Use of viral vectors, particularly adeno-associated viral (AAV) vectors, encoding appropriate growth promoting genes to transduce and genetically modify RGCs provides more long-term protection.[110]

Some regrowth of RGC axons across an optic nerve crush can be elicited after implantation of peripheral nerve fragments or MSCs into the vitreous, injuring the lens, or recruiting and activating monocytes/macrophages within the eye. Some regeneration is seen after blockade of cell-intrinsic molecules that suppress axonal growth, for example, after inactivation of Rho, a GTPase that inhibits growth cone motility (see Chapter 4 **Cellular signaling**), or by modification of relevant transcription factors and/or intracellular signaling pathways.[108,109] Overall, without combinatorial approaches that use several complementary therapies, adult RGC axons fail to regrow more than just a few millimeters past the injury site.

An approach that permits substantial long-distance regrowth of injured adult RGC axons has been to autograft PN segments from the same animal onto the cut optic nerve. Under these conditions, regenerating axons reinnervate target areas in the brain and some limited function restored. However, there is a functional cost involved in removing the nerves for transplantation. Alternatives are therefore being sought including nanotechnology approaches, matrices containing cultured OEG and chimeric PN grafts containing genetically modified Schwann cells.[111–113] Various types of hydrogel matrices, some containing peptide and aminosugar sequences, have also been used in attempts to promote the regeneration of RGC axons after more central optic tract injures in the brain.[114]

The optic nerve injury model provides an optimal system in which to examine combinatorial effects as pharmacological agents, recombinant growth factors or viral vectors can be injected into the eye to directly influence RGCs, while tissue and genetic engineering can be used to modify the environment within which

RGC axons regenerate. These interactions can be assessed at a single location using different combinations of factors, or at multiple locations—for example, testing the effect of factors applied to the cell body and supplying cells/appropriate factors near the growing tips of the axons.[116] Combined pharmacotherapeutic and vector-mediated strategies have been reported to drive more extensive regrowth of a proportion of RGC axons after optic nerve crush injuries, sometimes beyond the optic chiasm and at least as far as the optic tracts.[108] Note that, as in all CNS repair studies, great care must always be taken to distinguish between spared axons and axons that are truly regenerating after an injury.[117]

17.7 CNS: retina

The retina is a thin, layered neural structure consisting of diverse populations of RGCs, bipolar and amacrine cells, horizontal cells, photoreceptors (cones and rods), Müller glia, and retinal pigment epithelial (RPE) cells, the latter attached to a thin membrane called the Bruch's membrane (Fig. 17.9a). Bruch's membrane is partially synthesized by the RPE cells and contains laminin, collagen type IV, fibronectin, and heparin sulfate proteoglycans. RPE cells maintain the health and functionality of the photoreceptors. When the RPE and photoreceptors are detached from Bruch's membrane, photoreceptor cell death (and visual loss) ensues.

17.7.1 Diseases of the retina

Diabetic retinopathy, retinitis pigmentosa, and macular degeneration are three major diseases of the eye, while glaucoma—often associated with raised intraocular pressure—results in damage to the optic cup and retina. Age-related macular degeneration (ARMD) is the leading cause of blindness in the United States for people aged above 55 years. Current effective treatments for retinal disorders are limited, and a range of potential tissue engineering strategies exist.[118,119] These strategies often center around cell transplantation, from different sources and are sometimes accompanied by a scaffold or a matrix.

17.7.2 Cell transplantation

Intraocular cell transplantation is one therapeutic strategy for the treatment of degenerative diseases that primarily affect photoreceptors. Different transplantation methods (retinal sheets, fragments, dissociated mixed or purified cell suspensions) have been trialed, and different sources of cells have been used, including neural precursors, embryonic and postnatal retinal precursor cells, photoreceptors, neural stem cell lines, and bone marrow stem cells.[120–122] Fig. 17.9b shows how RGC-like cells are introduced to the eye/retina in an attempt to replace host neurons and/or repair optic nerve damage.

One tissue engineering strategy for ARMD is the transplantation of RPE cells to the subretinal space. A major challenge is that when RPEs are injected into the subretinal space as a suspension, they are unable to form ordered monolayers and eventually die. Cell transplantation substrates are therefore used as carriers to deliver cells, such as RPEs, iris pigment epithelium cells, and retinal progenitor cells to the retina (Fig. 17.9c). The seeded substrates are typically inserted subretinally through a slit in the sclera and choroid. Injected cell suspensions are exposed to high shear forces as they pass through the injecting needle, while therapies involving supporting substrates avoid such stresses. The use of cells on scaffolds for transplantation to the retina thus has advantages over injection of cells as spheres or single cell suspensions, where large numbers of cells do not survive the transplantation procedure. Many different transplantation substrates have been investigated, including natural and synthetic biomaterials, biohybrids,

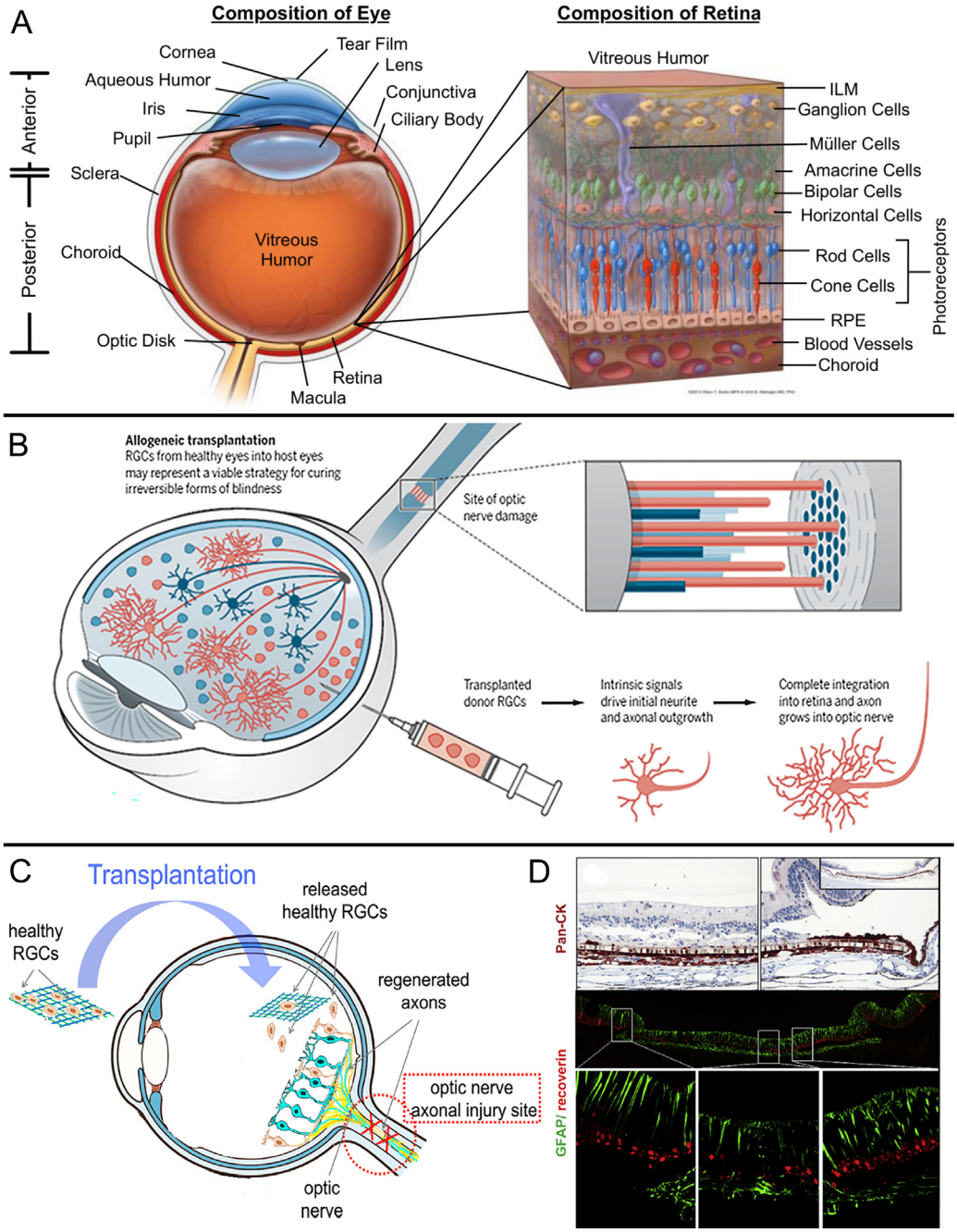

FIGURE 17.9

(a) Expanded view of the eye showing the layers of the retina. (b) Transplantation of injected cells into the eye and intended repair outcomes. (c) Transplantation of RPE-seeded scaffold for cell delivery. (d) RPE-seeded electrospun scaffolds transplanted subretinally in rabbits. *(a) From Parsons, D.E., et al., 2021. Peptidomimetics therapeutics for retinal disease. Biomolecules 11 (3), 339. (b) From Behtaj, S., et al., 2020. Retinal tissue bioengineering, materials and methods for the treatment of glaucoma. Tissue Eng Regenerat Med 17 (3), 253–269. (c and d) From Liu, Z., et al., 2014. Enhancement of retinal pigment epithelial culture characteristics and subretinal space tolerance of scaffolds with 200 nm fiber topography. Biomaterials 35 (9), 2837–2850.*

and various methods of microfabrication. Fig. 17.9d shows an example involving the implantation of electrospun sheets.[115] In this figure, RPE cells are brown, rod photoreceptors are red, and Müller glial cells are green. Substrates for subretinal implantation need to be a relatively thin thickness (the Bruch's membrane has an average of 5 μm) but require a certain amount of mechanical integrity to withstand the placement and implantation procedures. Research is ongoing to find an ideal and effective cell transplantation scaffold, including via 3D bioprinting,[123] for optimal delivery of stem cells or precursor cells.[119,124]

17.8 Future perspective

The injury response and healing mechanisms of the PNS are different to those of the CNS. The main challenge for PNS regeneration remains the reconnection of severed nerves that are separated from each other above a critical gap length. Fibers, cell transplants, and scaffolds continue to be investigated and some significant gap lengths are now being bridged with electrospun sheets. Oriented scaffolds with morphological similarities to structures within which the bands of Büngner form distal to the PN injury are often researched. Ultimately, the advances in scaffold fabrication processes such as 3D printing may provide the high-resolution scaffold needed for "fascicle-to-fascicle" guided regeneration. Any tissue engineering solution for the PN must improve on the widely used autograft surgery.

In situ gelling materials for the brain and spinal cord maintain their importance over the next decade for the delivery of therapeutics. Preformed or injectable hydrogels are often considered simple, while in fact they are highly complex, performing multiple roles required for treating the nervous system. These gels will perform increasing functions, including the survival/differentiation of transplanted cells and the delivery of NPs for targeted therapeutic delivery. Automated systems such as in bioprinting will continue to improve and allow more sophisticated matrix/bioink delivery.

The types of cell available for transplantation are also likely to be greater in the future, including perhaps activation of the patient's own endogenous neural precursor cells. The production of a successful regenerative bridging construct in the CNS requires knowledge of the dynamic temporal changes that occur after an injury, an understanding of the complex molecular and cellular environment in each injured area, and strategies to overcome this inhibitory environment. Combining cell transplantation in the contused spinal cord with other repair-promoting interventions such as pharmacotherapy and gene therapy allows substantially more "tools" for neurological recovery. Surgery can also be performed on transgenic animals (particularly mice), providing insights on the role of certain genes in regeneration with tissue engineered constructs. A major challenge will be to develop tissue engineering protocols that are suitable for use in chronic as well as acute injury situations. Further understanding of the effect of scaffold implantation will continue to provide tissue engineers with new concepts for bridging CNS defects and may one day provide an "off-the-shelf" solution to spinal cord repair.

The extensive use and positive safety data for mRNA delivery for COVID-19 vaccines will undoubtedly be directed toward therapeutics for the nervous system. Neural tissue engineering can play a central role in multitherapeutic delivery for combinations of nucleic acid delivery, cell transplantation, and drug release. The optic nerve is an excellent CNS model to test a combination of therapies, and this could become a model organ to investigate combinatorial therapies. In addition to the optic nerve, the retina is a tissue with surgical accessibility via the vitreous cavity. For this tissue, strategies in delivering polymeric sheets for cell transplantation to the retina will have important ramifications in the delivery of

tissue engineering strategies to the eye. Improved prosthetic devices that improve activation of the central visual pathways will also advance over the next decade.

More diverse neurological injuries and diseases are likely to be tackled with tissue engineering strategies. Approaches for the treatment of brain trauma and stroke are likely to involve combinations of therapies and may be distinct from therapies developed to treat degenerative diseases. Diseases such as multiple sclerosis and stroke may also respond to NP delivered drugs in clinical settings, particularly if systemic or intrathecal delivery can be a route for treating CNS injuries and disorders. The next decade should also see the translation of tissue engineering therapies to the clinic for treatment of injuries beyond the PNS. It is even possible that the long-time "holy grail" of assisting people suffering from SCI will be achieved in the next decade using some aspect of these bioengineering principles.

Summary

- Current approaches to successfully aid PN regeneration after PNI remain limited to a critical gap length, currently 30 mm for humans.
- Matrices and scaffolds have been explored to mimic an autologous nerve transplant, the typically used approach to promote nerve repair. The resulting nerve guides may be loaded with cells, functionalized with neurotrophic factors and/or combined with gene therapies.
- The inflammatory response that occurs after SCI may be managed using cell loaded or neurotrophic-functionalized scaffolds. Further, the use of inhibitors has also been employed to aid the inflammatory response. Hydrogels have been proposed as a minimally invasive strategy for their delivery.
- Nanomedicine via the use of NPs has been proposed as an approach to bypass the naturally occurring barriers to the CNS.
- Biofabrication techniques have developed throughout the recent years to produce matrices and scaffolds that better mimic the mechanical and physiological properties of native tissue for nerve regeneration.
- Regeneration of the optic nerve requires combinatorial approaches for sufficient RGC axonal regrowth. The optic nerve has been proposed as an ideal CNS model to test such combination of therapies.

Classical experiment

Schwann cell and OEG transplantation in P(AN-VC) nerve guides

The use of Schwann cell transplants within nerve guides for spinal cord repair was pioneered in the Bunge laboratory at The Miami Project to Cure Paralysis during the 1990s. Autologous Schwann cells can be isolated and cultured from peripheral nerves of the SCI patient. The potential of Schwann cell—filled nerve guides for spinal cord repair was demonstrated by Xu and coworkers,[53] who transplanted a poly(acrylonitrile-co vinyl chloride) P(AN-VC) tubular scaffold seeded with Schwann cells and Matrigel into the completely transected adult rat thoracic spinal cord. Several weeks after transplantation, myelinated and unmyelinated axons were present within the Schwann cell cable that connected the opposing spinal cord stumps (Fig. 17.10a—c). These and other experiments demonstrated the potential of using Schwann cells for spinal cord repair. A drawback is that responding axons grew across the Schwann cell graft but did not exit the graft to grow into the spinal cord distal to the implant, which

Classical experiment—continued

prevented the formation of new synaptic connections and, thus, new axonal circuits that are needed to control motor function. Increasing the level of neurotrophins just caudal to the SC transplant supports axonal growth beyond the implant. Fig. 17.10 visually depicts axonal growth across a Schwann cell-filled P(AN-VC) tube and neurotrophin facilitated exit of the axons in hemisection models. When there is Matrigel only (i.e., no Schwann cells) in the P(AN-VC) tube, there is limited growth of axons into the transplant. While Schwann cell inclusion with the Matrigel demonstrated axonal growth, the axons were only successfully guided out of the nerve guide via the delivery of BDNF and/or NT-3 into the caudal spinal cord a few millimeters from the implant.[54]

In the olfactory system, there is continued penetration of newly formed axons into the CNS, even in adulthood. OEGs enfold and guide these elongating olfactory axons so that the axons are unaffected by any growth inhibitory cues in the CNS environment. This unique feature of OEG was thought to be beneficial for eliciting axonal growth across and beyond an injury site. While OEG will ensheath axons, they do not myelinate them as is the case with Schwann cells. In pivotal experiments at The Miami Project to Cure Paralysis, OEG and Schwann cells were combined. A P(AN-VC) tube was filled with Schwann cells mixed in Matrigel and implanted into the adult thoracic spinal cord. OEGs were then injected into the rostral and caudal stumps. The results showed that the additional injections of OEG at either end of the Schwann cell channels allowed greater numbers of axons to exit the Schwann cell bridging grafts.[53,55] Descending axons such as those of the raphe system (located in the brainstem) grew in areas devoid of many Schwann cells but were seen in areas containing fibroblasts and OEG. Sensory axons also regenerated for long distances—up to 18 mm. Such an experiment demonstrates the utility of multiple treatments for a successful spinal cord repair outcome.

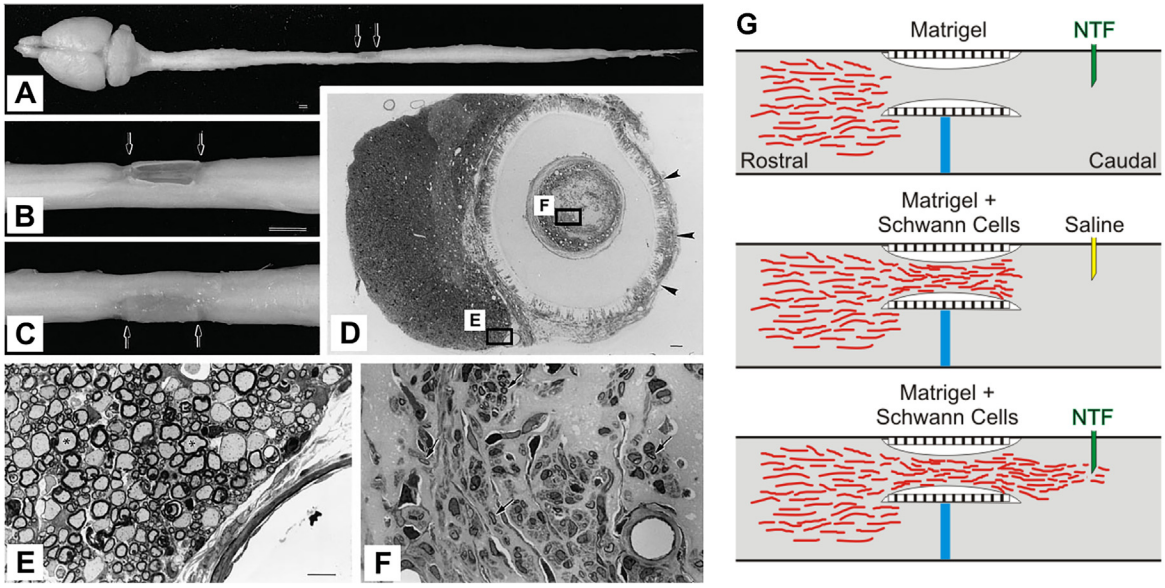

FIGURE 17.10

Schwann cell/Matrigel transplants in P(AN-VC) guides in a hemisected spinal cord. (a and b) Photographs of brain and spinal cord (a), and closer magnifications of the hemi-section and implant (b and c). (d) Transverse section depicting the tissue bridge and the intact contralateral spinal cord ventral and dorsal horn. (e and f) Myelinated axons (e) and smaller caliber axons (f) in the tissue cable. (g) Illustration of the effect of P(AN-VC) nerve guides implanted within hemisections. *(a—f) From Xu, X.M., et al., 1999. Regrowth of axons into the distal spinal cord through a Schwann-cell-seeded mini-channel implanted into hemisected adult rat spinal cord. Eur J Neurosci 11 (5), 1723—1740.*

State-of-the-art experiment

Bioprinting for the nervous system

The highly complex architecture of the nervous system requires cells to be accurately placed in a 3D environment when attempting to form structures that mimic neural networks. The automated and controlled placement of cells can be achieved by processing cell-laden biomaterials into a predesigned 3D scaffold with a technique called bioprinting, which has emerged as an area of focus for tissue engineering of the nervous system. Throughout this chapter, several examples in which bioprinting were applied to a particular tissue of the nervous system were introduced. The chronological development of such findings is summed up in Fig. 17.11.

In the first approach to bioprint nerve cells, primary hamster embryonic motor neurons were delivered onto a collagen gel-based biopaper (Fig. 17.11a).[79] The placement of these cells into a circular pattern using a modified Hewlett Packard desktop inkjet printer conferred the potential for high throughput and cost-effective way to position neural cells. The disadvantages of this technique include low ink cell density, discontinuous flow, and high working temperatures for thermal inkjet printer processing. Inkjet bioprinting may alternatively rely on piezoelectric forces, a setup which has been used to bioprint adult rat retinal ganglion cells and glia. Inkjet-based bioprinting is still restricted by height, which underlines the need to develop new techniques that would allow the fabrication of cell-laden constructs in a 3D environment.[80]

Neural cells were later incorporated into a hydrogel biomaterial-based ink (or simply, bioink) to develop 3D scaffolds in a layer-by-layer approach (Fig. 17.11b).[81] The 3D coculture of rat embryo neurons and astrocytes incorporated in collagen was important to demonstrate the possibility of coculture, which is relevant for the eventual establishment of neural networks in vitro given the range of cell types that make up neural tissue, and also the important interactions between neural and nonneural cells that naturally coexist in the body. This co-culture approach was later applied to RGD-modified gellan gum which incorporated primary cortical neurons into a brain-like structure printer layer-by-layer (Fig. 17.11b). The first approach to improve bioinks by combining different materials applied to the nervous system involved the extrusion of cell-laden fibrinogen into a thrombin solution, which led to the successful bioprinting of rat Schwann cells (Fig. 17.11c).[32]

In a first approach to coculture neural cells, human iPSC—derived neural progenitors were incorporated within an alginate and methyl cellulose scaffold matrix and successfully led to neural cell maintenance and proliferation (Fig. 17.11d).[82] In a separate study, cell-laden biomaterials were successfully tailored to the dimensions of the rodent spinal cord, demonstrating the ability to reengineer neural cell responses using digital light processing, a 3D printing technique also called microscale continuous projection printing (Fig. 17.11e).[83]

Development of other bioprinting techniques such as in gel/support bath printing has successfully been translated and optimized to neural applications throughout the years. Resorting to a gelatin support bath was initially explored in a cell-free approach to print cortical-like structures. More recently, this approach was paired with human neuroblastoma cells incorporated in a sodium alginate bioink, paving the way toward applying this technique to study neural interactions in bioprinted brain-like structures.[84]

Time-dependent structural transformation of bioprinted scaffolds adds a fourth dimension to bioprinting.[85] 4D approaches which incorporate external stimuli postprinting have recently been applied to advance the complexity of nerve guides. Nerve guides incorporating human MSCs were fabricated onto a reprogrammable multiresponsive graphene structure and have shown promising neuroregeneration ability after implanted in rats.[86] 4D-based bioprinting has further been proposed as a powerful tool for a more in-depth study of neuronal mechanical processes that occur in the cortical folds of the brain.[87]

Moving forward, bioprinted scaffolds will combine novel bioinks and drug-releasing polymers with 3D fine tailored microfluidic architecture to carefully engineer neural cell fate (Fig. 17.11f).[88] Taken together, the examples portrayed along the timeline shown in Fig. 17.11 illustrate the ever-developing field of bioprinting of nerve cell laden scaffolds toward multi strategic approaches of bioprinting for applications in the nervous system.

State-of-the-art experiment—continued

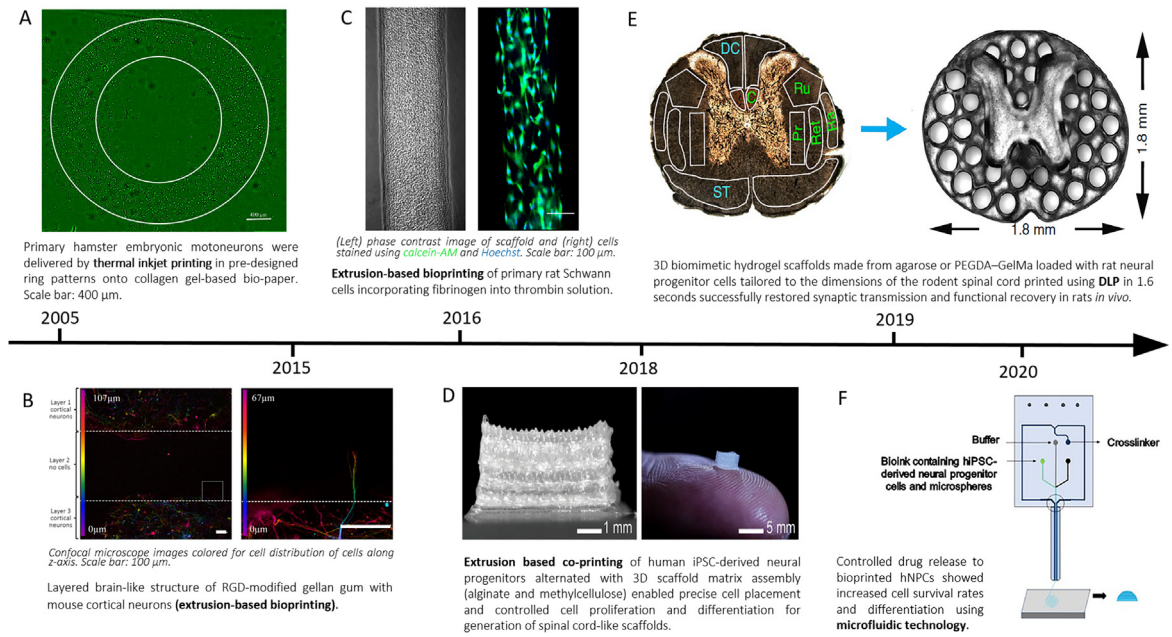

FIGURE 17.11

Selected examples of milestones through advancements in bioprinting applied to the nervous system. *(a) From Xu, T., et al., 2005. Inkjet printing of viable mammalian cells. Biomaterials 26 (1), 93—99. (b) From Lozano, R., et al., 2015. 3D printing of layered brain-like structures using peptide modified gellan gum substrates. Biomaterials 67, 264—273. (c) From England, S., et al., 2017. Bioprinted fibrin-factor XIII-hyaluronate hydrogel scaffolds with encapsulated Schwann cells and their in vitro characterization for use in nerve regeneration. Bioprinting 5, 1—9. (d) From Joung, D., et al., 2018. 3D printed stem-cell derived neural progenitors generate spinal cord scaffolds. Adv Funct Mater 28 (39). (e) From Koffler, J., et al., 2019. Biomimetic 3D-printed scaffolds for spinal cord injury repair. Nat Med 25 (2), 263—269. (f) From Sharma, R., et al., 2020. 3D bioprinting pluripotent stem cell derived neural tissues using a novel fibrin bioink containing drug releasing Microspheres. Front Bioeng Biotechnol 8, 57.*

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17.10 Assessment of your knowledge

(a) Answer the following questions to assess your command on terminology, facts, concepts, and theories learned in this chapter:

1. What is a main difference between sensory and motor neurons?
2. What is the role of Bands of Büngner?
3. Autograft approach is the "gold standard" to treat PNI when the gap between the damaged nerves is too large for the nerves to be reattached. List one disadvantage of the autograft approach.
4. Define critical gap length.
5. What is the approximate critical gap length for humans?
6. Once a PN nerve guide is sutured into position, an important protein naturally builds up due to the microenvironment response to the implant. What protein is this bridge made of?
7. Name four important neurotrophic factors in nerve regeneration.
8. Compared to PN, there is one main obstacle for regenerating axons in the CNS following injury to the spinal cord. Please identify this process.
9. What is one approach that could be employed to address the spinal injury scar that forms after SCI?

10. Animals are used to model different degrees of SCI. List one model used for SCI.
11. What is one main advantage of using NPs to treat SCI?
12. NPs, matrices, and scaffolds may be functionalized with peptide sequences that aim to promote and direct neuronal growth. List one.
13. Name one advantage and one disadvantage of using cats as animal models for SCI.
14. Cell transplants have been proposed as a therapeutic approach to treat SCI. Name one cell type.
15. What is the difference between 3D and 4D biofabrication?
16. Name one 3D printing technique applied to the nervous system.
17. A proposed alternative to bypass the blood–brain barrier (BBB) is to deliver drugs intranasally. Name two advantages of the intranasal delivery route.
18. Describe one way in which the visual system is a useful model to study regenerative success or failure after nerve fiber damage in the mature CNS.
19. One main goal after optic nerve crush is to enhance the survival of retinal ganglion cells (RGCs). Neurotrophic factors have been proposed, but what is a more long-lasting alternative?
20. Age-related macular degeneration (ARMD) has been tackled by transplanting retinal pigment epithelium (RPE) cells to the subretinal space.
    - 20.1. What is one main challenge when suspended RPEs are injected into the subretinal space?
    - 20.2. What is a proposed alternative to overcome this challenge?

(b) Answer the following questions to assess your ability to apply the concepts and theories learned in this chapter in real life, clinical, and scientific situations:

1. Current approaches to treat PNI include the use of nerve guides. Some commercially available nerve conduits degrade shortly after being implanted in the body (as little as 3 months), whereas others are designed to not degrade. What are the advantages and disadvantages of degradable (both short and long term) versus nondegradable nerve guides?
2. Describe how you would design an experiment that incorporates a multifaceted approach to tackle PNI.
3. How would a therapeutic approach differ when addressing the peripheral versus the central nervous system? (PNS vs. CNS)
4. Even within the CNS, the physical properties of the nervous tissue vary greatly in physical/mechanical properties. How do you expect to tailor the mechanical properties of scaffolds or matrices that are to be implanted in the brain versus the spinal cord, for example?
5. Injury to the spinal cord caused by trauma creates a local primary response after impact, which then triggers a cascade of secondary inflammatory responses. Outline the existing tissue engineering approaches that aim to tackle the later stages of SCI.
6. What are the main considerations when designing a therapeutic strategy for degenerative diseases versus trauma-related injuries to the nervous system?
7. Wallerian degeneration is a degenerative process that occurs after injury to an axon. Sketch a figure that compares a healthy axon to one undergoing Wallerian degeneration.

8. If you reflect on the concepts presented in this chapter, how would you argue the importance of having a team of multidisciplinary researchers and clinicians to develop new therapeutic approaches for nerve regeneration?
9. In your opinion, what is still a major challenge in tissue engineering of the nervous system?
10. What do you expect will be a major advance in the field of tissue engineering of the nervous system in the next 5–10 years?

Challenge-based learning

Designing nerve guides for pain management

Note for teachers: A challenge-based learning (CBL) user guide can be found at www.jandeboerlab.com/TissueEngineering with instructions and tips to run an effective CBL teaching session.

Background and vision

Pain is an essential human sensation to alert us to adverse conditions, but patients suffering from chronic pain have a much reduced quality of life, and in their situation, pain does not have an important signaling function. Pain management is a very active field of research and a current concept is that the immune system and peripheral sensory nervous system communicate by molecular signaling. When the nerve is severed in the case of amputees, painful neuromas form, however nerve guides appear to reduce the risk of this occurring.

Motivation and stakeholders

Pain sensitivity can be regulated/dysregulated by the immune system. Macrophages are able to promote and resolve pain sensitization and also T-cells play a dual role in pain sensitization. In addition to this, molecular therapeutic tools are available, such as small molecules, therapeutic antibodies, and siRNA, that can modulate the physiology of the immune system. It is therefore interesting to look into expanding on the opportunities to treat pain in amputees by using a tissue engineered implant. Future therapies should be based on molecular insight into the interplay between the immune- and nervous systems. Such therapies should be designed with the input from stakeholders such as intensive care unit doctors, nurses, physical therapists, mechanical engineers, immune engineers, and neural cell biologists.

Problem definition

Amputees suffer from painful neuromas that are regenerating nerves that are unable to find targets and bundle together.

Challenge

To develop a nerve guide that is specifically engineered to control nerve regeneration and prevent neuromas, potentially in conjunction with an external prosthetic.

Learning framework

Reading the Neural Tissue Engineering chapters and related literature will help you to understand the following:

1. Division, anatomy, and physiology of the peripheral nerve system
2. The sensory nerve system as part of the peripheral nerve system, and how the body feels pain.
3. The current state of peripheral nerve guides.
4. Conditions that exacerbate pain and how pain can evolve from acute to chronic.
5. The immune system as a pain mediator and the role of macrophages and T-cells.
6. Mechanisms of action of macrophages and T-cells to modulate pain.

For a more detailed look into your project, make a mind map summarizing the following:

7. Mechanism of action of current pain management drugs such as steroids and antiinflammatory drugs.
8. Specific dimensions of your nerve guide, for the different animal models.
9. The molecular signals that combine to stimulate or reduce nerve regeneration.

Challenge-based learning—continued

End product

A 3-min video explaining the solution of your challenge. Please include your motivation and the steps to execute your solution.

17.11 Glossary

Allogenic means taken from different individuals of the same species.

Alpha Motor Neurons are nerve cells, the cell bodies of which are found in the CNS, with axons that extend toward the periphery to innervate muscle and cause muscle contraction.

Apoptosis is a form of programmed cell death that occurs in multicellular organisms.

Autonomic nervous system controls the function of organs and glands; is separated into sympathetic and parasympathetic components.

Axon is a long, slender projection of a nerve cell that typically conducts electrical impulses known as action potentials away from the nerve cell body.

Axotomy refers to severing of an axon.

Biofabrication is the production of complex biologic products from living cells, matrices, biomaterials, and molecules.

Bioinks are carrier materials used to produce an engineered tissue using 3D printing.

Bioprinting is the utilization of 3D printing techniques to combine cells, growth factors, and/or biomaterials to fabricate biomedical parts, often with the aim of imitating natural tissue characteristics.

Blood–brain barrier is a highly selective semipermeable border of endothelial cells that prevents solutes in the circulating blood from nonselectively crossing into the extracellular fluid of the central nervous system where neurons reside.

Central nervous system (CNS) is the part of the nervous system consisting primarily of the brain and spinal cord.

Central pattern generator refers to neural circuits that produce rhythmic motor behavior without rhythmic input in activities such as walking or breathing.

Chemotactic is the movement of an organism or entity in response to a chemical stimulus toward the source of the stimulus.

Contusion injury is a tissue injury where the capillaries are damaged by trauma, causing localized bleeding that extravasates into the surrounding interstitial tissues.

Critical gap length is defined as a nerve gap over which no recovery will occur without the use of nerve grafting or bridging.

Critical-sized defects are defined as those that will not heal spontaneously within a patient's lifetime.

Distal refers to the (injured) part of the tissue away from the neuron cell body.

Donor site morbidity refers to complications and functional restrictions that the patient has to undergo because of harvesting tissue from a healthy donor site.

Fascicle-to-fascicle repair is a surgical technique to precisely match fascicles to recover nerve function.

Full transection is a complete interruption of white matter tracts, segmental gray matter, and associated nerve roots in the spinal cord.

Gamma Motor Neurons are nerve cells, the cell bodies of which are also found in the CNS, that take part in the process of muscle contraction by monitoring muscle length and stretch.

Glia are nonneuronal cells of various types that perform a wide range of support functions in the peripheral nervous system (PNS) and normal CNS.

Hemisection spinal cord injury (SCI) model is a tissue injury model characterized by damage to one half of the spinal cord.

In situ means the location where it occurs under normal circumstances.

Intrathecal space is the fluid-filled area located between the innermost layer of covering (the pia mater) of the spinal cord and the middle layer of covering (the arachnoid mater).

Microenvironment is the micrometer range environments of cells.
Minimal invasive surgery is a surgical technique that limits the size of incisions needed to lessen wound healing time, associated pain, and risk of infection.
Myelin is the insulation around axons that speeds up the conduction of nerve impulses.
Necrosis is unprogrammed cell death due to cellular damage or infiltration by pathogens, as opposed to orderly programmed cell death via apoptosis.
Nerve guides are conduits between the severed proximal and distal nerve stumps to provide structural and trophic support.
Neuropathological condition is a disease of the nervous system.
Neurotrophic factors are a family of biomolecules that support the growth, survival, and differentiation of both developing and mature neurons. Neurotropic factors are sometimes called neurotrophins.
Oligodendrocytes are a type of neuroglia whose main function is to provide support and insulation to axons in the central nervous system.
Parenchyma is the functional part of the tissue. In the nervous tissue, the parenchyma excludes, for example, fluid-filled spaces, blood vessels, or meningeal tissue (tissue that protect the brain and spinal cord).
Peripheral and Cranial Nerves contain nerve fibers (axons) that interconnect the central nervous system (CNS) to the periphery.
Peripheral nerve injuries (PNIs) occur when nerves of the peripheral nervous system are damaged due to physical or environmental factors or disease (e.g., accidents, falls, trauma, or diabetes).
Peripheral nervous system (PNS) consists of the nerves and ganglia outside the brain and spinal cord that reach organs and tissues like the heart, intestines, bones, and muscles.
Proximal is the (injured) part of the tissue closer to the neuron cell body.
Retrograde degeneration is a pattern of neuron destruction following axonal injury that spreads backwards along the axon, toward, and then into the nerve cell body.
Schwann cells are the myelin producing cells in the peripheral nervous system.
Sensory Neurons are nerve cells that carry sensory information from the body extremity to the CNS.
Spinal cord injury is damage to the spinal cord.
Spinal injury scar develops after spinal cord injury and consists of multiple cells and extracellular debris, with axonal growth inhibitory molecules to form a physical and chemical barrier for regenerating axons.
Wallerian degeneration is an active process of degeneration that results when a nerve fiber is cut or crushed and the part of the axon distal to the injury (i.e., farther from the neuron's cell body) degenerates.
Xenogeneic a tissue or organ that is derived from, originating in, or being a member of another species.

Some of these definitions were freely obtained and paraphrased from Wikipedia and Google.

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