Extracellular matrix-derived tissues for neurological applications

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6.1 Background

Nervous system injury or degeneration often results in debilitating and life-threatening conditions that may significantly limit the quality of life and life expectancy of afflicted individuals. In an attempt to facilitate nervous system repair and improve neurosurgical and functional outcomes, surgeons and researchers often strive to provide structural and proregenerative support via exogenous application of extracellular matrix (ECM) and/or ECM-derived materials. In general, these strategies are intended to structurally replace excised tissue, facilitate tissue regrowth, aid in hemostasis, and/or assist in the delivery of bioactive substances. ECM-based products offer several distinct advantages over synthetic materials. For instance, ECM-based products can be engineered to degrade over time while enabling controlled regrowth of new tissue. In comparison to treatments derived from nonbiologic sources, ECM-based products offer the advantages of decreased inflammatory and foreign body response. This is especially relevant in neurosurgical applications, where gliotic scarring and immune reactions remain primary obstacles to more widespread implant usage. Currently, neurosurgical applications of ECM-derived implants are targeted specifically to replace surgically removed or disrupted microstructure, thus providing a biological scaffold for regenerating neural cells to facilitate endogenous ECM deposition, and ultimately, tissue formation. Going forward, further research and novel products are being developed to improve these capabilities to treat a range of nervous system disorders. In this chapter we will discuss the current uses of ECM-based neurological implants and future directions in the development of restorative ECM-based biomaterials, constructs, and other implants.

6.1.1 Nervous system anatomy and function

The nervous system can be anatomically and functionally divided into the central nervous system (CNS) and peripheral nervous system (PNS). The CNS, relying on a

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specific macro and micro environment for function, is anatomically protected by the bony skull and spinal column and is immunologically isolated by the blood–brain barrier. Anatomically, the CNS is composed of the cerebrum, cerebellum, and spinal cord. The CNS functions in the production and transmission of efferent signals and the interpretation of incoming afferent signals from the PNS.

On a cellular level, the nervous system can be divided into the primary functional cells, called neurons, and the predominant support cells, known as glia. Neurons are composed of a cell body, dendritic processes responsible for processing incoming information, and an axon that transmits information to downstream neurons or end organs. There are several subtypes of neurons that are generally classified by anatomical region, neurotransmitter type, or based on inhibitory versus excitatory function. The roles of glia are quite diverse and crucial for nervous system function. The main glial cell types are astrocytes, microglia, oligodendrocytes, ependymal cells, radial glia, Schwann cells, and satellite cells. Glial cells are responsible for maintaining the microenvironment of the nervous system, with different types found in the CNS and PNS. In the CNS, astrocytes provide structural and functional support for neurons, playing a crucial role in diverse functions such as neuronal metabolism, signaling, and synaptic efficacy (Blackburn et al., 2009; Chen and Swanson, 2003). Also in the CNS, oligodendrocytes provide a myelin sheath around axons, with one oligodendrocyte responsible for multiple axons (Carlson, 2010). In the PNS this function is carried out by Schwann cells, with each cell responsible for the myelination of only a small segment of a single axon. In the CNS, neuronal cell bodies and glial cells make up what is referred to as gray matter, named for its gross appearance, whereas regions containing long axonal projections are termed white matter due to the appearance of the myelin sheathing. In addition, several other general cell types are associated with the neural vasculature and meninges, such as pericytes and fibroblasts.

In the healthy CNS, astrocytes are the most abundant type of glial cell and induce formation and maintenance of the blood–brain barrier, mediate nutrient exchange, and produce ECM components (Blackburn et al., 2009; Chen and Swanson, 2003). During development, astrocytes promote neuronal migration and axonal guidance through presentation of surface factors and secretion of growth factors permissive for growth and regeneration (Adcock et al., 2004; Joosten and Gribnau, 1989; Liesi and Silver, 1988). Following an insult to the CNS, astrocytes also take on a reactive phenotype and provide a physical and chemical barrier to sequester a region of focal injury, known as the glial scar (Pekny et al., 2014). The glial scar consists predominantly of reactive astrocytes, which become hypertrophied with interdigitized processes and deposition of inhibitory ECM factors, resulting in an environment unsuitable for regeneration (Fawcett and Asher, 1999). Following injury and/or focal degeneration, microglia in the CNS also become activated to scavenge dead/dying cells and other debris to clear the site of injury (Hernandez-Ontiveros et al., 2013). These cells, along with other glial support cells, play a key role in maintaining nervous system function and reestablishing homeostasis following an insult.

In contrast, Schwann cells and satellite cells are the resident glia in the PNS, and are responsible for the maintenance of ionic gradients and the production and maintenance of insulating myelin sheaths (Bhatheja and Field, 2006). Schwann cells also play a key role in regeneration, ECM production, and signaling (Bhatheja and Field, 2006). Peripheral nerves are in effect bundles of axonal projections carrying both efferent and afferent information. Outgoing efferent neurons, such as motor neurons, have cell bodies residing in the spinal cord whereas afferent neurons (sensory) have cell bodies located just outside the spinal cord in the dorsal root ganglia. In both cases the cell bodies are protected by bone of the spinal column and the meninges.

Although intricately connected, both functionally and anatomically, the CNS and PNS have distinctly different extracellular environments due to the unique demands of the specific functional components. While the PNS and CNS are markedly different on a cellular level, from a surgical perspective several unifying principles exist. The constituent parts of CNS and PNS are encapsulated in several layers of connective tissue sheathing, serving to protect fragile neural tissue, and in the CNS, maintain the fluid-filled subdural compartment. The meninges, the connective tissue layers of the CNS, are comprised of three layers, termed the dura mater, the arachnoid mater, and the pia mater. The dura mater is the tough, watertight outer layer, composed of a double layer of interwoven collagen fibers (Protasoni et al., 2011). The arachnoid mater, named for its composition of a web-like network of collagen fibers, contains microvasculature and spans the space between the dura and the brain or spinal cord. The pia mater composes the capsular covering of the brain and spinal cord. On a microscopic level, the subpial space separates the meninges from the outermost covering of the brain parenchyma, the glia limitans. Composed of astrocyte foot processes, the glia limitans functions in maintaining the blood–brain barrier along with vascular endothelial cells and their tight junctions (Kettenmann and Ransom, 2005; Shearer and Fawcett, 2001). The glia limitans and the blood–brain barrier serve as both a physical and an immunological protective barrier, maintaining the integrity of the cerebral microenvironment (Kettenmann and Ransom, 2005; Shearer and Fawcett, 2001). Nerves in the PNS are also encapsulated in further layers of connective tissue, chiefly composed of collagen fibers. As a peripheral nerve exits the spinal canal, the dura and arachnoid mater come together to form the outer layer of nerve sheathing, known as the epineurium. Each individual myelinated nerve axon is covered in endoneurium, with axon bundles held together by perineurium. Several bundles and associated vasculature constitute a nerve held together by the thicker fibrous epineurium (Lee and Wolfe, 2000; Lundborg, 1987) (Fig. 6.1).

From a surgical perspective, reconstitution or replacement of the connective tissue layers is critical—in the CNS to maintain the fluid-filled environment, and in the PNS to maintain nerve continuity and facilitate regrowth. Similarly, due to relatively small operative spaces and sensitivity of both the CNS and PNS to direct mechanical compression, surgical hemostasis is of paramount importance. In both connective tissue repair and surgical hemostasis, ECM-derived products play a pivotal role.

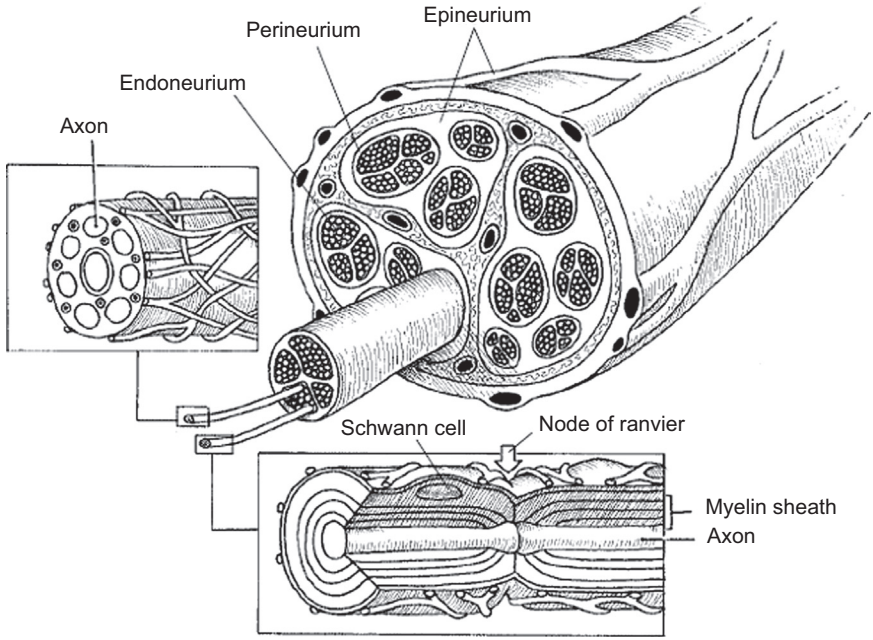

Figure 6.1 Anatomy of a nerve. Nerves consist of a modal structure, comprised of an axon that is surrounded by a membrane called the endoneurium. Several of these axons comprise a fascicle, which is bounded by the perineurium. In turn, numerous fascicles make up the peripheral nerve, which is covered by the epineurium. The inset on the left shows an unmyelinated axon and the inset on the bottom shows a myelinated axon (Lee and Wolfe, 2000). Reproduced with permission from Elsevier; Lundborg, G., 1987. Nerve regeneration and repair. A review. Acta Orthop. Scand. 58, 145–169.

Thus, the nervous system relies on an infinitely complex microarchitecture and cell interaction schema to regulate the complex function of the human body. While still an area of intense study and development, the current understanding of the nervous system has propelled groundbreaking research spanning basic neuroscience, neural engineering, and clinical neurology and neurosurgery. Indeed, the proper development of the nervous system relies on precisely controlled cellular and axonal guidance while sustaining normal function of the nervous system is based on the continued maintenance of these complex networks. A significant aspect of development and maintenance relies on specialized support cells and a distinct ECM environment. Consequently, ECM plays a crucial role in surgical repair and regenerative medicine efforts.

6.1.2 Role of ECM in development

ECM plays a pivotal role in cell migration, axon/process elongation, differentiation, and synapse formation mainly through providing structural and trophic support (Franco and Muller, 2011; Plantman, 2013; Rowlands et al., 2015; Soleman

Table 6.1 Major roles of nervous system extracellular matrix (ECM) molecules (Barros et al., 2011; Reichardt and Prokop, 2011)

	Migration	Axon outgrowth	Differentiation	Synapse Formation
ECM Components	Fibronectin Laminin Tenascin Thrombospondin Hyaluronic acid Collagens Reelin	Fibronectin Laminin Tenascin Thrombospondin	Fibronectin Laminin Tenascin Thrombospondin	Laminin Tenascin Hyaluronic acid Collagen IV

et al., 2013). ECM components act as a roadmap for the developing nervous system, anchoring neuronal and glial networks after formation, and providing the microenvironment for synaptic transmission (Barros et al., 2011). Since ECM molecules have such a vital role in the creation of neural structures and function, many researchers have focused their efforts to understand and utilize these traits for therapeutic benefit. As described in more detail in subsequent sections, Table 6.1 presents a summary of the most abundant ECM constituents in the CNS and PNS and their major roles. For the sake of simplicity, not all functions of the molecules are stated.

In order to appropriately direct cell migration, axon outgrowth, cell differentiation, and/or synaptogenesis, ECM molecules are expressed in a spatial as well as temporal manner in the nervous system. Certain molecules are specific to either the CNS or PNS. During CNS development, Reelin, an ECM molecule associated with neuronal migration, is secreted by specific cells in the laminated brain, and binds to lipoprotein receptors that are expressed by migrating neurons and radial glia cells (Barros et al., 2011). Conversely, Reelin expression is significantly lower in the PNS, and is only expressed in developing Schwann cells, with a sharp decrease in expression in the mature PNS (Panteri et al., 2006). Additionally, ECM molecule expression can vary depending on the location or function of the particular area within the nervous system. For example, in the CNS, hyaluronic acid (HA) is the most abundant ECM component, but fibrillar proteins, such as collagens, laminin, fibronectin are more prevalent in the basal layer, subpial space, and blood vessels in addition to white matter tracts (Dwyer and Matthews, 2011). In contrast in the PNS, collagen and laminin are more prevalent, whereas HA expression is limited and concentrated within the myelin sheath (Eggli et al., 1992). The temporal dependence of ECM expression is also notable, with higher levels of laminin found during development, but diminishing with maturity in the CNS (Reichardt and Tomaselli, 1991). In addition to a decrease in laminin as the CNS matures, levels of HA have been seen to decline as well. This is believed to correlate with the reduced ability of the mature CNS to regenerate. However, decrease in ECM molecules following

peripheral nerve injury (PNI) is not believed to be as severe, preserving the ability to regenerate following PNI (Huebner and Strittmatter, 2009). Therefore, researchers have continued to study ECMs during development in an effort to learn how to circumvent regenerative limitations of the adult nervous system by providing proregenerative ECM constituents.

6.1.2.1 Cell migration and process outgrowth

Cell migration and axonal outgrowth are complex processes necessary for the establishment of appropriate neural connections. During development, stem cells in the periventricular zone are the progenitor cells for future CNS structures. Differentiating cells must migrate along a network of guiding cells and ECM to reach their targets. Radial glial cells, progenitor cells that give rise to several cell types, exhibit processes extending from the periventricular zone to the cortical plate. These processes serve as a scaffold along which immature neurons migrate to their final destination in the cortex. HA, laminin, fibronectin, and thrombospondin are believed to be the major ECM molecules involved in promoting neuronal migration and axonal outgrowth along with collagen, specifically collagen IV (Barros et al., 2011; Franco and Muller, 2011; Huebner and Strittmatter, 2009). These ECM molecules, including chondroitin sulfate proteoglycans (CSPGs), such as aggrecan (Carulli et al., 2005), are distributed throughout various layers and facilitate migration to the cortical plate, with the exact mechanism still largely unknown (Barros et al., 2011). Laminin, thrombospondin, and other ECM proteins are secreted by astrocytes in the CNS. Furthermore, ECM proteins such as laminin and perlecan are present in the basal layer of the neocortex. Radial glia end feet interact with these molecules in the basal lamina through integrin (several β and α integrins) and dystroglycan receptors (Barros et al., 2011), promoting neuronal migration and differentiation. Additionally, thrombospondin type 1, which is known to promote neurite outgrowth, is present along white matter tracts, and promotes oligodendrocyte migration, has been seen to be produced by immature astrocytes during development (Christopherson et al., 2005; Osterhout et al., 1992; Scott-Drew and Ffrench-Constant, 1997). Following injury, specifically spinal cord injury, reactive microglia, macrophages, and oligodendrocytes have been seen to induce synthesis of ECM molecules that promote axon growth, such as tenascin-C, keratan sulfate, and CSPGs (Hausmann, 2003). Similar ECM molecules (laminin, thrombospondin, etc.) are also secreted by Schwann cells in the PNS to mediate axonal guidance and cell migration (Jones and Bouvier, 2014).

6.1.2.2 Differentiation

Aside from providing physical or structural cues for cell migration and axon pathfinding/growth, ECM molecules such as fibronectin, laminin, tenascin, and thrombospondin play a significant role in differentiation of progenitor cells into the correct phenotype. For example, laminin modulates neurotrophic factors and affects cellular differentiation. Similarly, tenascin regulates oligodendrocyte differentiation with

the type of tenascin present determining whether differentiation of oligodendrocyte progenitor cells is promoted or inhibited (Barros et al., 2011; Garwood et al., 2004; Pesheva et al., 1989).

6.1.2.3 Synaptic formation

The term synapse refers to the space between a neuron and an end-target receiver (neuron or muscle end plate) across which signal transduction occurs via electrical and/or chemical (neurotransmitter exchange) means. Synapses rely on a specific microenvironment, maintained through modulation of ionic homeostasis and neurotransmitter metabolism. If the ionic gradients in the synaptic cleft are disrupted or appropriate neurotransmitter clearance/recovery does not occur, synaptic transmission is inhibited. Here, ECM proteins support the maintenance of the mechanical structure of the synapse. Synapses are surrounded by a network of glyco-proteins, specifically CSPGs, that are released by nearby neurons and astrocytes that help maintain the synapse as well as transfer signal across the synaptic cleft. In the CNS, the ECM is chiefly comprised of HA that wraps around the neuron or processes at the synapse to form a perineuronal net (PNN) functioning to maintain the synapse (Celio et al., 1998). The PNN is also composed of ECMs including CSPGs (eg, neurocan, versican, brevican, aggrecan, etc.) and tenascin (Fig. 6.2). Additionally, glial cells, such as astrocytes, play a major role in synapse formation, and secrete thrombospondin to help modulate synaptogenesis. It should be noted that the ECM molecules implicated for synaptogenesis in the CNS vary over time as the CNS develops (Barros et al., 2011).

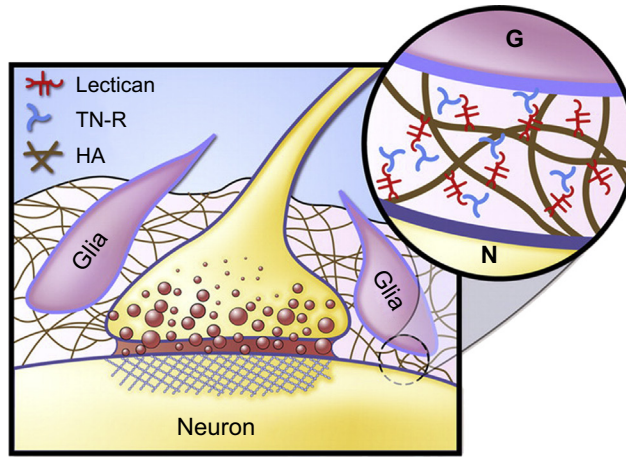

Figure 6.2 ECM molecules help modulate synapse formation in the central nervous system. Hyaluronic acid wraps around neurons and neurites to form a perineuronal net, while Lectican and Tenascin-R are secreted factors that promote synaptogenesis.
Reproduced with permission from The American Physiological Society; Sykova, E., Nicholson, C., 2008. Diffusion in brain extracellular space. Physiol. Rev. 88, 1277–1340.

In the PNS, the neuromuscular junction (NMJ) is an interface between neurons and the muscle end plate responsible for initiating muscle contraction. Studies have found that NMJs are embedded in basal lamina, which is composed of laminin, heparin sulfate proteoglycans (HSPGs), CSPGs (ie, versican, brevican, neurocan), and collagen IV, the most abundant ECM molecule at the NMJ synapse (Barros et al., 2011; Patton, 2003).

6.1.3 Nervous system regeneration and the role of ECM

Regeneration of nervous system structures after injury is a complex process, requiring an appropriate balance of factors to limit the extent of injury while providing a pro-regenerative environment. Axotomized neurons undergo a process called Wallerian degeneration, in which the distal nerve degenerates, leaving the target denervated and unable to function. Regeneration of the nervous system requires the reestablishment of axonal pathways. Successful axonal regeneration necessitates a microenvironment permissive for regeneration to enable axonal growth through a chemical and, in the CNS, a physical barrier to the appropriate target. Successful regeneration of axonal connections is intimately dependent on the ECM environment, since scarring, chemotaxis, and microarchitecture of axonal projections is predicated on ECM secretion and function. Due to the fragility of the nervous system, as well as the duality of the factors present after injury in their ability to promote or inhibit neuroregeneration, a delicate balance is necessary to successfully regenerate nervous tissue. This can prove to be a difficult feat in the adult nervous system.

6.1.3.1 Central nervous system

Following the generation of neuronal populations and long-distance axonal pathways during development, microenvironmental changes in the CNS favor stability over regeneration (although local sprouting and synaptic plasticity remain). This is partly driven by a shift in the extracellular microenvironment from progrowth ECM constituents during development to antigrowth constituents when mature. Specifically, numerous ECM proteins promote neuronal migration and axonal pathfinding during development (eg, laminin, fibronectin); however, alternative ECM molecules (eg, CSPGs, certain isoforms of tenascin, etc.) act to inhibit migration and axon outgrowth once a cell and/or axon has reached the appropriate target (Carulli et al., 2005; Galtrey and Fawcett, 2007).

Moreover, the CNS is exquisitely sensitive to damage due to its inability to replace lost neurons and regenerate damaged or severed axonal connections. Following focal injury, brain or spinal cord tissue may undergo substantial macro- and microenvironmental changes, potentially resulting in astrocyte “reactivity,” immune cell activation, and the formation of a glial scar surrounding the site of focal injury (Fawcett and Asher, 1999; Kawano et al., 2012; Silver and Miller, 2004; Yuan and He, 2013). The formation of a glial scar—consisting primarily of hypertrophied, reactive astrocytes—initially serves to sequester regions of widespread cell death and inflammation, therefore limiting the extent of the primary lesion. However, glial scar tissue

remains beyond the initial injury phase, persisting as a physical and chemical barrier for nervous system regeneration. Specifically, the reactive astrocytes forming the glial scar secrete CSPGs (eg, neurocan, phosphacan), which are potently inhibitory for growth cone extension and may even induce growth cone collapse (Jones et al., 2003; McKeon et al., 1999; Zimmermann and Dours-Zimmermann, 2008). Moreover, other inhibitory molecules have been found to be upregulated following CNS injury, including Nogo, myelin associated glycoprotein (Whitlock et al., 2009), and oligodendrocyte-myelin glycoprotein (OMgp) (Barros et al., 2011; Galtrey and Fawcett, 2007; Huebner and Strittmatter, 2009). The increase in inhibitory CSPGs and other molecules are believed to be major contributors to the decreased ability of the CNS to regenerate following insult. Thus, the mature CNS does not provide a suitable environment for neuronal migration and long-distance axonal pathfinding following injury and/or neurodegeneration.

6.1.3.2 Peripheral nervous system

In contrast to the CNS, the PNS possesses a greater intrinsic ability to regenerate. Despite the onset of Wallerian degeneration, neurons projecting peripheral axons (ie, dorsal root ganglia (DRG) neurons and spinal motor neurons) are able to enter a proregenerative state to elicit axon regrowth to distal targets provided a sustained permissive environment and trophic support. Consequently, nerves that have undergone crush injury and maintain an intact epineurium fare better than severed nerves. In the PNS, Schwann cells provide regenerative support following injury. Schwann cells are present in the basal lamina of the PNS, where following axonal degeneration they revert to a proregenerative state and increase secretion of numerous factors (eg, nerve growth factor, brain-derived neurotrophic factor, glial-derived neurotrophic factor) that promote axon regrowth and guidance. Schwann cells also form long tubes in the endoneurium known as bands of Büngner that secrete ECM components such as fibronectin, laminin, and other growth factors to aid in axonal regeneration (Gao et al., 2013). In addition, Schwann cells help to remodel NMJs by extending and retracting processes from the junction, thus influencing axonal reinnervation following injury (Kang et al., 2014). These proregenerative features of Schwann cells in the PNS are not exhibited by glial cells in the CNS, and are a critical feature that differentiates the regenerative capability of the PNS versus the CNS. However, there are limits to the regenerative abilities of the PNS, as segmental nerve defects greater than several centimeters in length and injuries closer to midline necessitating many months to years for regenerating axons to reach distal targets are still generally considered “unrepairable” (Hoke, 2006). Here, ECM-based products in clinic and under experimental development are being used to bolster the innate regenerative abilities of the PNS.

6.1.4 Benefits of ECM scaffolds for neurological applications

Based on the myriad of functions that ECM plays in the development and maintenance of the nervous system, it is an important biomaterial and tissue engineering substrate for implant development. Depending on the desired outcome, implants

with a pro- or antiregenerative phenotype may be developed based on a particular ECM, and may be suitable for such varied applications as facilitating neural regrowth or sealing the meningeal compartment. In either mode, the function of ECM is to provide three-dimensional (3D) structure, serve as substrates for cell/process infiltration, and, in some cases, provide trophic support to cells and tissues.

The structural functions of ECM include various mechanical properties to support the particular application. When used in the creation of neurological implants, ECM can be manipulated to provide the necessary level of stiffness or flexibility to support the appropriate cell–implant interaction. For example, increased viability was seen when neurons were cultured on substrates of modulus similar to that of intact brain (Georges et al., 2006). This may be a result of the interdependence between matrix stiffness, pore size, and pore density of the ECM substrate, which not only contributes to its mechanical and tensile properties, but also affects cell adhesion, proliferation, and neurite outgrowth (Cullen et al., 2007; Cullen et al., 2011; Georges et al., 2006).

In addition, the importance of geometric properties, such as surface curvature, is also being exploited to promote neural regeneration (Cullen et al., 2008; Smeal et al., 2005; Smeal and Tresco, 2008; Wen and Tresco, 2006). For example, axons in culture were shown to have enhanced longitudinal growth along microfibers with diameters similar to that of axon fascicles, likely due to the mechanics of minimizing process bending (Smeal et al., 2005). Additional surface features such as scratches, ridges, and grooves can aid in guiding neurite outgrowth, as these may be similar to aligned fibers presented by ECM molecules such as collagen (Withers et al., 2006). Interestingly, the shape of the substrate affects neuron morphology and neuritogenesis as well. When neurons were cultured on varying shapes, their cytoskeletons reformed to imitate the shape of the substrate (Jang and Nam, 2012). Therefore, the physical and mechanical properties of a substrate can greatly impact neuronal somatic and neurite behavior, and can be used to manipulate outgrowth. Since neurons have been seen to respond optimally to physical/mechanical properties similar to those in healthy in vivo conditions, such as those provided by native ECM, the use of ECM to promote neural regeneration is a promising strategy for neuroregeneration.

Along with physical support, the trophic functions of ECM promote interactions between molecules and cells to direct cell development and behavior. Specifically, numerous structural cell adhesion molecules/binding sites along ECM interact with the specific cell types to promote neural development and regeneration. The primary family of cell–matrix adhesion receptors is known as integrins, whereas common cell–cell adhesion molecules include neural cell adhesion molecule (NCAM) and L1. In addition, interactions between ECM and neurotrophic factors can influence neurotrophic factor concentrations by binding to ECM and/or modulating degradation, and in many cases result in synergistic action affecting numerous cell signaling cascades. Such signaling has been shown to influence differentiation, migration, and/or proliferation of specific cell types. Neurotrophic factors such as nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic

factor (BDNF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) have been shown to act in concert with various ECM constituents in promoting regeneration (Reynolds and Woolf, 1993; Sofroniew et al., 2001). For instance, factors such as FGF are known to bind to heparin sulfate proteoglycans of the ECM, which help protect it from proteolysis (Reynolds and Woolf, 1993). Additionally, VEGF, which plays a key role in angiogenesis, has been seen to increase nerve regeneration following PNI in rodents. VEGF is present in various isoforms, some of which remain diffusible, while other isoforms bind to HSPGs and form a chemoattractive gradient around VEGF secreting cells (eg, during development: neuronal precursor cells) that lead to vessel sprouting and ultimately promote tissue growth (Rosenstein et al., 2010). The ability of ECM proteins to interact with cells and tissue make them especially desirable as scaffolds for neural repair. In turn, adhesion to host structures and integration into the host microenvironment promotes implant function.

ECM plays a crucial role in endogenous neural tissue development and regeneration, and thus is advantageous as an exogenous treatment to affect a desired regenerative outcome. For medical use in tissue engineering and regenerative medicine applications, ECM is generally acquired as naturally synthesized cellular products in vitro or through decellularization of harvested tissue ex vivo. The lack of cells present in these constructs is often seen as an advantage for regenerative medicine, generally resulting in a decreased host inflammatory response, thus reducing the risk of graft rejection. Although beyond the scope of the current chapter, the benefits of including living cells in tissue-engineered constructs has been noted, including the creation of living scaffolds to promote regeneration by guiding neural cell migration or axonal pathfinding as well as structurally and functionally replacing neural circuitry (Struzyna et al., 2014, 2015). Here, the desired cell types are often undifferentiated stem cells or immature neural cells, and can be added to the ECM-derived construct, which could be host-derived cells (eg, induced pluripotent stem cell (iPSC) derived) to minimize immune response.

6.2 Current applications of ECM-derived products in CNS surgery

CNS surgery requires access to the most biologically isolated parts of human anatomy. Access to the CNS involves disruption of the skin, underlying connective tissue, bone, dura mater, arachnoid and pia mater, and dissection through brain parenchyma in certain cases. The effort often disrupts neuronal connections and glial networks. Currently, repair of CNS structures is not a viable neurosurgical approach, and ECM-based neurosurgical implants currently in use are aimed at repairing iatrogenic damage to CNS during the surgical intervention or replacing removed or damaged meningeal structures. While nervous system repair is exceedingly complex, the ideal approach to surgical intervention would utilize implants designed to repair the damaged nervous system.

6.2.1 Dural repair products

Dural repair products are ubiquitous in neurosurgical operating rooms. During surgery, dura is often removed or damaged, either directly from surgical technique or due to the pathology being treated. As a result, primary dural closure may be challenging at best and impossible at worst. Therefore, dural replacement strategies are crucial to successful neurosurgical intervention. The ideal graft would pose minimal infection risk, reconstitute the dural layer in a watertight fashion if necessary, be immunologically inert, and exhibit ease of handling and application. To these ends, both synthetic and autologous options have been used extensively. While certain products are intended purely as an onlay graft, others are denser and have higher tensile strength, tolerating being sutured into the patient's dural edge to facilitate watertight closure. Cerebrospinal fluid (CSF) leak through a dural defect is a potentially disastrous complication, leading to a higher infection risk and need for further surgical intervention. Therefore, the choice of dural closure method is paramount to a successful operation.

The most attractive dural repair mechanism is the use of the host's own tissues, chiefly pericranium, a connective tissue layer overlying the skull. While the immunologic and structural advantages of an autograft are obvious, the harvest of the tissue can prove problematic during surgery, requiring extensive dissection away from the surgical site and increased risk of morbidity. The potential lack of a pericranial source adjacent to the surgical site, and an increase in operative time further complicate tissue harvest. Additionally, ossification of pericranial flaps has been reported (Hoover and Mahmood, 2001). For these reasons, most cranial cases requiring dural replacement have utilized commercially available dural substitutes—they are conveniently available in several sizes, can be cut to the needed shape, and are quickly applied (Zerris et al., 2007) (see Table 6.2). Animal-based heterologous grafts, mostly of porcine, equine, and bovine origins, are used extensively. While synthetic dural substitutes exist, the majority of surgeons prefer the more popular ECM- or collagen-based products because of their superior biocompatibility, decreased foreign-body response, and reported lower infection rates (Malliti et al., 2004).

Animal-derived ECM- or collagen-based products currently used are mainly derived from bovine connective tissue, such as the Achilles tendon, fetal tissue, and pericardium. Porcine and equine products are also available. The connective tissue is treated through company specific processes involving decellularization and subsequent lyophilization for either a compact or loose fiber composition. During lyophilization, the collagen is chemically separated, dehydrated, and freeze-dried for further processing of the purified protein. The collagen-based dural repair products primarily use collagen I fibers with some also including collagen III, as these are abundant in the nervous system.

In addition to animal-derived collagen grafts, plant-derived cellulose-based products are also available (Fig. 6.3). The cellulose-based dural repair product Synthecel (DePuy, West Chester, USA) has been shown to perform functionally equivalent to collagen-based grafts in surgical outcomes (Rosen et al., 2011). The cellulose-based graft offers high tensile strength, with a superb ability to hold suture. Also, because of its cellulose composition, it does not adhere to host tissues making reoperation and

Table 6.2 Commercially available dural onlay products

Product name	Manufacturer	Composition	Source	Notes
DuraGen Plus Matrix	Integra Life Sciences	Collagen I	Bovine Achilles tendon	Fully resorbable at rate of tissue growth, porous
DuraGen XS	Integra Life Sciences	Collagen I	Bovine Achilles tendon	Higher collagen density, greater tensile strength
Durepair	Medtronic	Collagen I and III	Fetal bovine	Highly porous, 10–100 μm pores
DuraMatrix	Collagen Matrix	Collagen I	Bovine Achilles tendon	6–9 month resorption time; available as onlay and as suturable membrane
Lyoplant Onlay	Aesculap	Unspecified	Bovine pericardium/bovine split hide	Biologic, bi-layer membrane allows for adhesion to native dura
Lyoplant	Aesculap	Collagen 1 matrix	Bovine pericardium	Pure collagen, freeze-dried for loose fiber composition, allows for ingrowth
Duraform	Depuy-Synthes	Collagen I	Bovine	Improved wet handling characteristics
DuraMatrix	Stryker	Collagen I	Unspecified	
Synthecel	Depuy	Cellulose	Biosynthesized by *Gluconacetobacter xylinus*	Nonadhesive Biosynthesized cellulose and water
Lyomesh	Audio Technologies	Unspecified	Equine pericardium	

separation of layers more facile. Conversely, the cellulose grafts do not interact with ingrowing dura. The cellulose used in the production of Synthecel is produced by the bacteria *Gluconacetobacter xylinus* (Rosen et al., 2011). Since the source is biosynthesized and not animal derived, theoretically the risk of transmission of infection to the graft recipient is lower, although this has not been elucidated in clinical trials.

Additionally, the use of hemostatic onlay patches, for example TachoSil (Baxter, Deerfield, USA), over dural suture lines has been reported with equivalent results to conventional dural closure techniques. Consisting of equine collagen embedded with

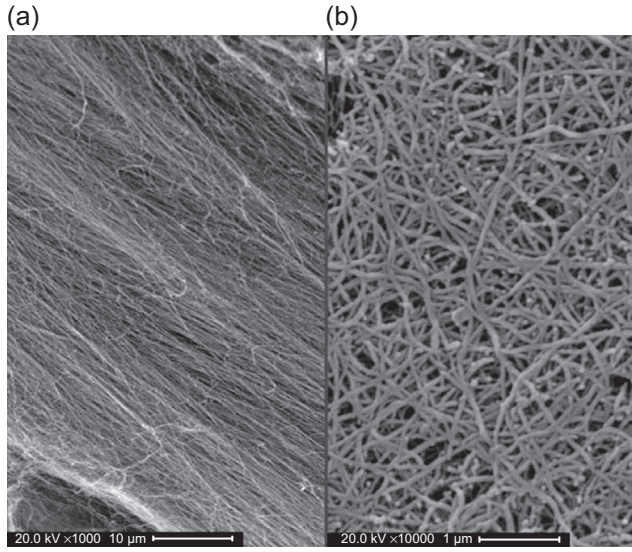

Figure 6.3 Structure of cellulose-based dural repair product. Scanning electron microscope image of (a) side view, scale: 10 µm, and (b) top view, scale: 1 µm, of biosynthesized cellulose showing a multilayered structure.
Reproduced with permission from Wolters Kluwer Health, Inc.; Rosen, C.L., Steinberg, G.K., Demonte, F., Delashaw Jr., J.B., Lewis, S.B., Shaffrey, M.E., Aziz, K., Hantel, J., Marciano, F.F., 2011. Results of the prospective, randomized, multicenter clinical trial evaluating a biosynthesized cellulose graft for repair of dural defects. Neurosurgery 69, 1093–1103 discussion 1103–1104.

fibrinogen and thrombin, the onlay patch creates an adhesive clot over the dural suture line. Theoretically, this additional onlay and clot layer reinforces dural closure. It has also been reported as a closure option for small dural sinus injuries (Gazzeri et al., 2015). Similarly, the use of this combination collagen/fibrin product has been reported with promising results in transsphenoidal surgery, an operation with a high risk of CSF leak (Hong et al., 2015).

The choice of dural substitute is mostly based on availability and surgical experience, with neurosurgeons preferentially using products that they are familiar with and are used to handling. Nevertheless, ECM-based grafts offer several advantages. Collagen-based products are porous, allowing for fibroblast ingrowth and host ECM deposition, and are absorbable at the rate of tissue growth. However, the increased biocompatibility to host tissue also results in meningocerebral adhesions and can be problematic in reoperations. Similarly, early graft dissolution has been reported (Abla et al., 2010; Narotam et al., 1995).

Of note, human-derived dural replacement products became popular in the 1960s, employing freeze-dried cadaveric dural grafts. However, these allogeneic grafts presented additional risks as serious infections were reported, including prion (eg, Creutzfeld–Jakob) disease and lentivirus transmission (Centers for Disease Control and Prevention, 2008; Walcott et al., 2014), and thus were discontinued from use.

Currently, the infection risk in cranial surgery associated with the use of dural substitutes is controversial (Abu Hamdeh et al., 2014; Sabatino et al., 2014). A risk of chemical meningitis, the exact etiology of which is unclear, has been reported with the use of dural substitutes; however further investigation into this phenomenon is needed. Although self-limiting and managed with high dose steroids, it has been reported to result in the need for reoperation and graft removal (Moskowitz et al., 2009; Parker et al., 2011). This complication is especially pertinent in posterior fossa surgery, where the dural graft is continuously bathed in CSF (Parker et al., 2011).

6.2.1.1 Adjuncts to dural repair

In procedures where the risk of CSF leak is high, neurosurgeons will often use adjuncts to dural patching in order to maximize the chances of a watertight closure. While some overlays such as Surgicel (Ethicon, Somerville, NJ, USA) (discussed in the next section) are used to create a blood patch-like barrier, tissue glues have gained significant popularity as a secondary dural closure measure. Fibrin glue such as Tisseel (Baxter, Deerfield, IL, USA), is applied with an aprotinin, a fibrinolysis inhibitor, and thrombin. The combination is sprayed over the suture lines of the dura–dural patch interface to reinforce the closure. Duraseal (Medtronic, Baton Rouge, LA, USA), another popular method, involves the simultaneous application of polyethylene glycol and trilysine (an amino acid with reactive end groups). The combination forms instantaneous cross links and functions in the same manner as the fibrin glue. Aside from providing a watertight closure, tissue glues also function in promoting hemostasis. Although extremely popular, tissue glues are not without risk, with case reports of neural element compression from large clumps of tissue glue as well as neurotoxicity (Epstein, 2010).

6.2.2 Hemostatic aids

The cranial vault, unlike other anatomic spaces, is a confined space with no ability to expand in times of injury or edema. Surgical intervention is often aimed at evacuating a space-occupying lesion, be it a hematoma or a tumor that is causing compression of vital neural structures. Similarly, because of the microsurgical nature of most CNS interventions, clarity and visibility in the surgical field is of paramount importance. The same principles are applied to surgery of the spinal cord. Moreover, bleeding in the subarachnoid space is avoided at all costs as the inflammatory response and irritation of the brain leads to complications and creates challenges in the postoperative course. Consequently, any surgical intervention in the CNS relies on appropriate hemostasis—the tight confines of the intracranial space dictate a low tolerance for even minor surgical bleeding. In modern neurosurgery, ECM-based hemostatic aids play a key role in achieving hemostasis and continue to be a vital part of successful neurosurgical procedures (see Table 6.3).

ECM-based hemostatic aids function in both active and passive ways. Collagen or cellulose networks aid in platelet adhesion and organized clot formation. For instance, collagen-based products have gained popularity due to their postulated superior

Table 6.3 Commercially available collagen hemostatic products

Product name	Manufacturer	Composition	Notes
Vitagel	Stryker	Microfibrillar collagen and thrombin	
TachoSil	Baxter	Equine collagen and fibrinogen and thrombin	Combines topical thrombin/plts/collagen
Instat MCH	Ethicone	Microfibrillar collagen	
Avitene MCH	Bard	Microfibrillar collagen	

hemostatic properties through activation of the clotting cascade and platelet adhesion through binding of glycoprotein VI (Al-Tamimi et al., 2012). They may also serve as delivery vehicles for hemostatic products such as thrombin and fibrinogen. Whether the hemostatic aid is aimed as a delivery vehicle, a scaffold, or both, dictates its function during surgery. Several commercial options exist for ECM-based hemostatic onlays. The most popular variants, due to their frequent use over several decades, are gelatin based. While gelatin is derived from collagen, it is in hydrolyzed form and therefore does not have the bioactive properties of collagen (Liou et al., 2013).

6.2.2.1 Hemostatic sponge

Hemostatic sponges are a staple of neurosurgical operating rooms, and while certain products are solely gelatin based, some sponges contain a combination of collagen and gelatin (see Table 6.4). They are packaged in dry form and can be molded to the desired size. Typically, the sponges are cut into small sections and soaked in thrombin solution for topical application. Collagen and gelatin hemostatic sponges efficiently control venous bleeding through a combination of approaches—direct pressure, providing a collagen network for clot formation, and delivering topical thrombin. Multiple variations exist commercially, with both bovine and porcine collagen sources, as well as some products combined with gelatin for maximal effectiveness.

The use of hemostatic sponges is not without caveats. A well-described complication is hemostatic sponges left in the surgical field swelling up and causing local mass effect on neural structures. For these reasons, while the sponges are fully absorbed over time, when operating in very small, confined cavities it is recommended to remove the sponge after hemostasis is achieved. The risk of infection is theoretically increased with any foreign body implant, collagen sponges not excluded. While several products emerging on the market offer pretreated sponges with antibiotics (gentamycin), the use of such products has not gained significant popularity since the risk of infection with the use of standard collagen sponges is extremely low.

It should be noted that collagen sponges offer an ideal local drug delivery mechanism. Their porosity and absorptive properties make them well tailored for topical application of substances during surgery. While this has been classically taken advantage of for delivery of hemostatic agents, other bioactive substances have also been

Table 6.4 Commercially available collagen hemostatic sponges

Product name	Company	Product	Source
Genta-Coll	Resorba	Collagen sponge with gentamycin	
Kollagen Resorb	Resorba	Collagen sponge	
Helistat	Integra Life Sciences	Collagen sponge	Bovine Achilles tendon
Gelfoam	Pfizer	Collagen sponge	Porcine skin

used in this fashion. For example, the application of recombinant human bone morphogenic protein (rhBMP) in spine surgery employs collagen sponges for local drug delivery (Friess et al., 1999).

6.2.2.2 Oxidized regenerated cellulose

Oxidized regenerated cellulose has been commercially available since the 1960s, with great success as a hemostatic aid in several surgical subspecialties including neurosurgery. The production process converts pure plant-derived cellulose into a grid-like fabric. The cellulose is oxidized to enable more effective clot formation (Emilia et al., 2011). A representative product is Ethicon's Surgicel product line, which offers several options in cellulose based hemostatic aids. Surgicel Original has been used extensively in many surgical subspecialties including neurosurgery. This knitted sheet of cellulose fibers is meant to be applied directly to a bleeding surface. Touting quicker hemostasis and versatility, several new products with greater absorbability, bactericidal properties, and faster hemostasis have been developed with great success. Surgicel Snow, Fibrillar, and NuKnit (suturable) offer different density of fibers, consistency, and handling characteristics.

A popular use of Surgicel (Somerville, NJ, USA) and like products is to line the surgical cavity at the end of an intraaxial surgery, for example, lining the cavity after a craniotomy for tumor resection. While extremely effective for hemostasis, this practice occasionally proves problematic due to the ring-enhancing appearance of Surgicel on MRI and should be taken into consideration in postoperative imaging and specifically in an evaluation for possible infection. Similarly, Surgicel granuloma have been reported, mimicking residual or recurring tumors on postoperative imaging (Menovsky et al., 2011; Sandhu et al., 1996).

6.2.2.3 Particalized hemostatic agents

Particalized hemostatic agents offer several advantages over sheet-like onlays. In neurosurgery, hemostatic matrix in combination with thrombin has gained increasing popularity. The variants currently in use in neurosurgery are composed of gelatin granules mixed with thrombin. The matrix is applied to surfaces or surgical cavities, with resultant swelling of the granules and combined chemical and mechanical hemostatic effects.

Microfibrillar collagen hemostats (MCH) are available for use in neurosurgical procedures from several manufacturers. The base component is the ubiquitous purified bovine collagen. Packaged in a flour consistency, MCH powder can be applied to irregularly shaped surfaces, is fully absorbable, and has been shown to be very effective in controlling bleeding. MCH with thrombin combinations are also commercially available. Similar to thrombin and gelatin particle products such as Surgiflo (Ethicon, Somerville, NJ, USA) and Floseal (Baxter, Deerfield, USA), the MCH particles and thrombin are combined in a syringe with an applicator tip for ease of use. Popular in other surgical subspecialties, these products are not yet used in neurosurgery as the dissemination of particles in the CSF and in the subarachnoid space is thought to lead to further inflammation, mechanical blockage of granulations, and further deleterious effects. Particalized hemostats, when used appropriately, are extremely effective hemostatic aids and offer the neurosurgeon versatility and ease of use. However, particalized hemostats can sometimes prove precarious in neurosurgical procedures. If used with a large caliber bleeding source such as a torn dural sinus, the particles can disseminate intravascularly and cause vessel occlusion and potentially severe complications. Similarly, the particles can deposit in the subarachnoid space.

6.3 Current applications of ECM-derived products in the PNS

The endogenous regenerative ability of the PNS has allowed for the generation of products to augment regeneration and has resulted in tremendous advances in the management of nerve injury. In cases where the nerve ends can be surgically reanastomosed without introducing undesirable tension, ECM-based and/or synthetic implants are currently employed to protect and mechanically reinforce the anastomosis site to increase the likelihood of a successful repair. In the case of segmental nerve defects, due to severe trauma, tumor, or scar tissue removal, the gold standard for surgical repair is an autologous nerve graft (autograft) in which a sensory nerve from another part of the body is used to provide a bridge for regenerating axons to cross the lesion (Pfister et al., 2007, 2011). This method requires an additional surgery site, leading to unnecessary trauma, loss of function of harvested nerve (ie, loss of sensation), and may lead to donor site morbidity and neuroma formation. Thus, in some cases synthetic and/or ECM-derived nerve guidance tubes (NGTs) may be used as a substitute for the autograft to provide a bridge connecting the proximal and distal nerve stumps. The purpose of these NGTs is to direct regenerative processes, including Schwann cell infiltration from both the proximal and distal stumps and axonal regrowth from the proximal stump, as well as to prevent lateral infiltration of scar-forming cells that may impede or block nerve regeneration. However, all NGTs currently on the market are vastly inferior to the autograft, and thus, in general, are only used clinically for short nerve lesions (eg, <3 cm) in noncritical sensory nerves close to the end target (eg, repair in the wrist for reinnervation in the hand). In this space, there are several NGTs currently available (see Table 6.5), with ECM-based NGTs generally fabricated using

Table 6.5 Commercially available collagen nerve tubes

Product name	Manufacturer	Composition	Notes
Neuroflex NeuroMatrix	Collagen Matrix; sold by Stryker	Type I collagen	Comes in flexible and stiff tube varieties
NeuroMend	Collagen Matrix; sold by Stryker	Type I collagen	Collagen wrap for nerves in continuity
NeuraGen	Integra Life Sciences	Type I collagen	Stable over 3–4 years in animal studies
NeuraWrap	Integra Life Sciences	Type I collagen	Semipermeable inner membrane allows passage of small molecules but prevents escape of growth factor; outer membrane prevents scar ingrowth

collagen. These ECM-based NGTs may retain bioactive properties, lacking in synthetic materials, that support cell infiltration and regeneration. Further development of ECM-based NGTs is intended to reduce the need for autologous nerve grafts.

6.3.1 ECM-based implants in peripheral nerve surgery

Peripheral nerve surgery is aimed at decompression of entrapped nerves, repair of damaged or severed nerves, or rerouting healthy nerves to denervated muscles in order to increase functional use. When surgery involves establishing new connections between nerves or repairing a damaged nerve, regardless of the indication, the success of peripheral nerve repair relies on an efficient, protected anastomosis. Although the autograft remains the gold standard for peripheral nerve repair, NGTs and acellular nerve allografts (ANAs) aimed at improving nerve regeneration and repair are gaining efficacy and therefore popularity due to the drawbacks of autografts. These disadvantages include paucity of available nerve tissue in the body, donor site morbidity, increased chances of infection, and greater recovery time as a result of the nerve harvesting procedure. In addition to the mechanical protection of regenerating axons, ECM-based implants assist in the guidance of growing axonal projections towards their target (De Ruiter et al., 2009; Pfister et al., 2007). To this end, several surgical implants have been developed to guide axonal projections across injury sites and protect the anastomosis from biochemical and mechanical effects of surrounding tissues.

6.3.1.1 Acellular nerve allografts (ANAs)

An emerging technique of peripheral nerve repair utilizes acellular ECM conduits produced from donor peripheral nerves, or ANAs. Although techniques differ across brands, the allografts are prepared through a decellularization process (lyophilization, irradiation, detergent processing, etc.) while preserving the ECM

structure of the nerve to allow for effective axonal guidance and structural support (Moore et al., 2011; Myckatyn and Mackinnon, 2004). Axogen Avance (Chalahua, FL, USA), a representative ANA currently in use, is manufactured using a proprietary decellularization process and is available in multiple lengths for convenient grafting. In both experimental models and pooled clinical analysis, ANAs have been found to perform better than nerve guidance tubes alone and have begun to approach the efficacy of the gold standard, autograft (Tang et al., 2013; Whitlock et al., 2009). A proposed mechanism for the observed superiority of ANAs to NGTs is increased axonal distribution across the graft, attributed to the ANA's endoneurial microstructure (Johnson et al., 2011). Currently, several groups are investigating approaches combining ANAs with cellular and growth factor delivery (Accioli-De-Vaconcellos et al., 1999; Zhang et al., 2014; Zhu et al., 2015). With further studies needed for optimization, ANAs remain an attractive alternative to autografts.

6.3.1.2 Nerve guidance tubes (NGTs) and wraps

NGTs are generally employed where a portion of a nerve is missing or must be excised due to extensive damage. The tubes are used to bridge the gap between the proximal and distal ends of a severed nerve and in animal studies have been shown to perform equivalent to end-to-end anastomosis for short lesions. NGTs serve to guide axonal projections in a more organized fashion and prevent extraneural extension of growing axons (Konofaos and Ver Halen, 2013). Classically, silicon tubes have been used for nerve guidance and protection in PNS surgery (Konofaos and Ver Halen, 2013). However, the use of silicon alone proved problematic due to a fibrotic host response, poor nutrient transfer, and the need to invasively remove the scarred tissue at a later date. NGTs manufactured from other synthetic materials such as poly(glycolic-acid) (eg, NeuroTube, Synovis, St. Paul, MN, USA) or bovine collagen (Neuromend, Neuromatrix, Stryker, Kalamazoo, MI, USA; Neuragen, Plainsboro, NJ, USA) have provided a solution to problems inherent to silicon NGTs. These NGTs have the added advantage of porosity, allowing nutrient and neuroactive substance exchange (De Ruiter et al., 2009). They are available in several different configurations and diameters; certain experimental products include an inner collagen matrix and microtube constructs for assistance in nerve ingrowth (Fig. 6.4).

Nerve wraps are a similar product, consisting of a collagen type I tube that is cut longitudinally and can be placed around a damaged nerve that remains structurally intact. While some products like Neuromend (Stryker, Kalamazoo, MI, USA) are pure collagen, others are synthesized from biologic tissues directly; Axoguard (Axogen, Alachua, FL, USA) is manufactured from ECM derived from porcine intestinal submucosa. These NGTs and wraps are rigid enough to protect the repaired nerve from mechanical compression, while retaining flexibility to move with natural limb movement. Like all collagen products, they are fully absorbable over a time frame of 3–6 months, with some studies suggesting the long absorption time can lead to late-stage nerve compression. Overall, the inflammatory reaction to current NGTs in clinical use has also been reported to be minimal (Nectow et al., 2012).

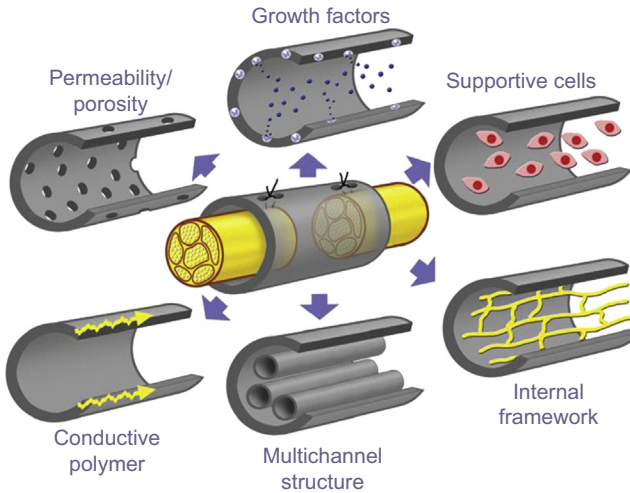

Figure 6.4 Modified nerve guidance tubes. Conventional hollow nerve tubes are modified by optimizing porosity, inclusion of growth factors and supportive cells, incorporating an internal framework and multichannel structure, or using a conductive polymer to enhance nerve outgrowth and regeneration (De Ruiter et al., 2009).
Reproduced with permission from Elsevier; Hudson and Schmidt (1999).

In an effort to promote successful regeneration by more closely mimicking the natural ECM, researchers have combined several ECM-derived molecules within NGTs. One such example is implantation of a silicone NGT filled with the ECM molecules collagen, laminin, and fibronectin into a rat peripheral nerve injury model. Here, a majority of the animals treated with laminin-fibronectin exhibited regeneration that bridged the nerve lesion as well as increased nerve diameter and myelination, whereas only a little over half of control animals treated with empty silicone NGT exhibited regeneration (Chen et al., 2000). In another study, collagen I NGTs filled with laminin- and fibronectin-coated collagen fibers were transplanted into a peripheral nerve injury model. Post-transplantation, an increase in the number and myelination of regenerating nerve fibers was seen in the group treated with the ECM-coated fibers, as compared to the control groups consisting of uncoated collagen fibers (Tong et al., 1994). Moreover, the collagen elements degraded by 30 days post-transplantation, and the new nerve tissue was surrounded by epineurium (Tong et al., 1994). Increased regeneration in the fibronectin-laminin coated grafts further suggested that the interplay of laminin, fibronectin, and collagen promotes more successful neuroregeneration than collagen alone. Additionally, an NGT comprised of collagen I and poly(ε-caprolactone) (collagen/PCL) was found to elicit electrophysiological outcomes and muscle innervation was similar to that of autografts following repair of sciatic nerve gaps in rats (Yu et al., 2011). Collectively, these results suggest that NGTs containing ECM constituents may be more effective in nerve regeneration than empty NGTs alone. As an example, a novel commercially available construct on the market, Nerbridge (Toyobo, Osaka, Japan), is an NGT

composed of a PGA tube filled with a novel collagen core, intended to improve nerve growth and promote angiogenesis.

While current collagen-based NGTs and wraps effectively mitigate problems inherent to earlier generation implantable products (which exhibited a high incidence of scarring and fibrosis, acting as major obstacles for nerve regeneration), they do not yet perform near the level of the autograft. The parameters of the NGTs themselves have been manipulated extensively, with different thickness, mono- or multichannel structure, and tube wall porosity. In addition to collagen tubes, NGTs made from rolled fibronectin mats have also been investigated. Various studies have also looked at the inner filler of NGTs, including both ECM-based and cellular contents.

Although these NGTs may provide the backbone for the next-generation of nerve repair technology, including aligned ECM support, proregenerative factors, and as necessary, preseeded endogenous or exogenous cells, an increasing number of NGTs are under development using a combination of stem cells and growth factors. Gene therapy and protein delivery techniques have also been explored (Johnson et al., 2008; Pfister et al., 2007). While most experimental strategies remain inferior to autografts with regard to the level of functional recovery achieved, some strategies show promise for equivalence across short nerve lesions. While no clear superior technique has been established, research is ongoing with promising results showing lower inferiority to peripheral autograft.

6.4 Future and emerging applications of ECM technology for neurological therapies

Neural degeneration due to injury or disease may lead to a significant loss of neurons and/or axonal pathways. Common secondary mechanisms responsible for this phenomenon include increased oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammatory response, among many others. The development of implants aimed at alleviating damage and facilitating more robust regeneration is gaining traction as a potential intervention for classically irreversible and devastating neurodegenerative diseases or insults.

6.4.1 Neuroprotective scaffolds

In order to prevent or slow disease progression or secondary injury, neuroprotective measures are being developed to decrease cellular damage and slow neuronal death. Most commonly, these measures are focused on developing constructs comprised of molecules and materials that function to protect against oxidative stress, excitotoxicity, and the host inflammatory response. One such ECM used for neuroprotection is fibronectin. In general, fibronectin works by binding to heparin I, fibrin, collagen, and chondroitin sulfate, and as discussed previously, plays a significant role in neuronal migration and axon outgrowth during development (Alovskaya et al., 2007). Recently,

fibronectin peptides (fibronectin V) and fibronectin mats have been delivered into injury models affecting the striatum and spinal cord of rats. After injection into the striatum, a greater number of dopaminergic neurons and a decrease in expression of inflammatory markers and apoptotic cells was observed (Duan et al., 2000). When fibronectin was delivered following spinal cord injury in rats, decreased apoptosis, lesion size, and axonal damage was observed, while hindlimb locomotor performance was enhanced (King et al., 2010). Proposed mechanisms, while not well elucidated, include a decrease in leukocyte infiltration and binding and activation of α5β1 integrin receptor leading to downstream activation of neuroprotective Bcl-2 (Tate et al., 2007). Additionally, when fibronectin was applied to a focal brain ischemia model in the rat brain, leukocyte infiltration was not significantly altered; however, a decrease in apoptotic cells and infarct size was observed, as well as an increase in functional outcome (Zhao et al., 2005). In contrast to the CNS, fewer efforts have been made to slow the effects of secondary injury in the PNS.

6.4.2 ECM-derived regenerative scaffolds

Biologically inspired materials are being vigorously pursued to promote neuroregeneration. Some of the most common materials used in this pursuit are abundant in the neural ECM, both during development and after maturation. These ECM materials include, but are not limited to, collagen, fibronectin, laminin, and HA. Due to their structural integrity, these molecules can be used either as the base of a scaffold or as coatings to modify biologically inert scaffolds, such as laminin (due to limitations in 3D structure of laminin-only ECM).

6.4.2.1 Collagen

Collagen is the most abundant component of the PNS ECM and plays a significant role in its maintenance. As such, collagen is the primary component of several NGTs available clinically. Furthermore, collagen is extensively incorporated into tissue engineered nerve grafts. Researchers exploit the ability of collagen fibers to direct neurite extension and/or cell migration, and have found that the orientation of collagen fibers significantly impact migration and neurite extension (Liu et al., 2012). Studies have shown that when cultured on aligned collagen I fibers, DRG neurons and astrocytes orient themselves in the direction of fiber alignment (as opposed to random growth on nonaligned fibers) (Alberti et al., 2014). In addition, glial fibrillary acidic protein (GFAP) expression, which is upregulated in reactive astrocytes forming the glial scar, was seen to decrease when astrocytes were cultured on aligned collagen nanofibers as compared to controls. In vivo studies in which aligned and nonaligned electrospun collagen nanofibers were rolled to form a scaffold and transplanted into an acute rat spinal cord injury model, found that the aligned fiber scaffolds retained more robust structural stability. Furthermore, GFAP-positive astrocyte accumulation was not found within the lesion site when aligned scaffolds were transplanted, suggesting that aligned collagen nanofibers are promising for promoting nervous system regeneration (Liu et al., 2012).

More recent studies have improved upon previous nanofibrous conduits by eliminating the need to roll 2D aligned nanofibers. Here, collagen is incorporated into synthetic, inert materials to make the conduit more durable, while retaining bioactive properties contributed by the collagen. For example, a nanofiber conduit comprised of collagen and poly(lactic-co-glycolic acid) (PLGA) has been developed. When these conduits were implanted into a 13 mm rat peripheral nerve injury, they were seen to promote axon regeneration, myelination, action potential propagation, neuromuscular transmission, and functional recovery when compared to nonaligned conduits, albeit at levels below those attained by autografts (Ouyang et al., 2013).

6.4.2.2 Fibronectin

Fibronectin, which also plays a significant role in cell migration, is incorporated into nerve guidance tubes to promote regeneration. For example, freeze-dried silk/single walled carbon nanotubes were enhanced by electrospinning fibronectin nanofibers onto the nanotubes. Not surprisingly, with the addition of fibronectin nanofibers, the conduits were found to be bioactive, as proven by the viability of glioblastoma cells cultured on the fibronectin-loaded silk nanotubes in vitro (Mottaghitalab et al., 2013). When the fibronectin-loaded silk conduits were transplanted into a 10-mm rat peripheral nerve injury, greater functional recovery was observed in comparison to silk conduits alone (Mottaghitalab et al., 2013). In another study, a nanosilver-embedded collagen I–based scaffold that was coated with ECM was developed and transplanted into rodent PNI models. The scaffold was coated with either laminin only or laminin and fibronectin. Both scaffolds (laminin only and laminin- and fibronectin-coated) performed comparable to autografts in restoring nerve function following injury (Ding et al., 2011). However, when scaffolds coated with laminin only were transplanted, partial regeneration was seen as the laminin could not induce nerve growth like the bands of Büngner, which secrete fibronectin as well as laminin (Ding et al., 2010). Therefore, when scaffolds coated with both ECM molecules were transplanted, a significant increase in nerve regeneration and functional recovery was observed (Ding et al., 2011).

6.4.2.3 Hyaluronic acid (HA)

Although collagen, laminin, and fibronectin are present in the CNS, they are widely implemented in conduits for peripheral nerve repair. Since HA is the most abundant ECM molecule in the CNS, it is utilized largely in applications for CNS repair and regeneration. Furthermore, HA boasts high biocompatibility and porosity, making it suitable for promoting neural regeneration. Studies have found that when HA hydrogels were coated onto cortical injury sites in rats, glial scarring and the number of $GFAP^+$ cells were significantly decreased surrounding the lesion site (Lin et al., 2009). In another study, HA blended with collagen I supported robust neuronal differentiation (Brannvall et al., 2007). Finally, treatment with HA–laminin scaffolds resulted in a decrease in the number of GFAP-expressing astrocytes and an increase in process outgrowth in vivo, both of which are necessary for successful regeneration in the CNS (Wang et al., 2012).

Following neurodegeneration, the concentration of growth factors and other proteins may remain suboptimal for effective regeneration, resulting in inhibited or decreased cell migration, differentiation, and/or axon pathfinding. In this case, physical support alone from ECM-derived conduits may not suffice to promote successful regeneration. For this reason, ECM-derived scaffolds have been fabricated by employing various techniques to be used as vehicles for the delivery of appropriate growth factors, cells, and/or other agents.

6.4.2.4 Growth factor presentation

Several growth factors are known to promote neuroregeneration through axon extension, cell migration, or differentiation. These factors include NGF, basic fibroblast growth factor (bFGF), glial-derived neuotrophic factor (GDNF), and CNTF. If these factors are simply injected into the injury site, they generally diffuse into the surrounding body fluid or tissue and do not remain at the site of injury where they are necessary. Therefore, ECM-based scaffolds and delivery vehicles are employed to provide sustained release of such factors at the injury site. Recent studies have investigated the binding of neurotrophic factors to scaffolds in order to promote and direct regeneration. For example, CNTF and bFGF were bound to a collagen-binding domain (CBD) that was attached to a collagen scaffold. Collagen scaffolds functionalized with CBD-CNTF, CBD-bFGF, or CBD-CNTF, and CBD-bFGF were transplanted into a 35-mm facial nerve gap in minipigs. It was found that the collagen functionalized with both CBD-CNTF and CBD-bFGF exhibited more extensive nerve regeneration and myelination, as well as improved nerve function (Cui et al., 2014). In a similar study, CNTF was bound to a laminin modified collagen scaffold in order to concentrate CNTF at the injury site. This was done by fusing a laminin binding domain (LBD) to the N-terminal of CNTF; therefore, the LBD–CNTF would bind specifically to the laminin modified collagen (Cao et al., 2011). This method successfully increased the concentration of CNTF available at the injury site, leading to enhanced axon growth and regeneration, as well as functional recovery (Cao et al., 2011).

Another common method of growth factor delivery is the use of microspheres. For example, in the CNS, PLGA microspheres loaded with VEGF and BDNF were utilized. The microspheres were then distributed in HA prior to gelation. An initial burst release of the growth factors from the HA hydrogel–PLGA microspheres was seen, followed by a steady release rate (Wang et al., 2011). Systems similar to this show promise for controlled release of growth factors to promote successful and more efficient nerve regeneration.

6.4.2.5 Cell transplant vehicle

Neurodegenerative disease and neurotrauma may lead to a significant loss of cells. Therefore, in order to promote regeneration, it may be necessary to replace and/or replenish lost neural cells. Cells can act to maintain a proregenerative environment by directly interacting with the host milieu and modulating the concentration of proregenerative factors through feedback from the surrounding tissue. For these reasons, the inclusion of cells in scaffolds can prove to be extremely advantageous. ECM-based

scaffolds or vehicles are effective for cell transplantation due to their biocompatibility, cell-protective, and cell adhesion properties. Since transplantation of mature cells may lead to an exacerbated host immune response, immature cells or stem cells are frequently chosen to seed scaffolds utilized for regeneration. In a recent study, a laminin-coated chitosan scaffold seeded with bone marrow stem cells (BMSCs) was transplanted to repair a 10-mm peripheral nerve lesion. While newly formed nerve cells covered the interior of the conduit even in the absence of BMSCs, the group treated with BMSC-seeded conduit displayed increased regeneration as evidenced by increased nerve regrowth, muscle mass, functional recovery, as well as decreased neuronal death, inflammatory, and fibrotic responses (Hsu et al., 2013).

Along with stem cells, Schwann cells also exhibit a neuroprotective effect in the PNS. Therefore, preservation of Schwann cells may prove beneficial in achieving successful neural regeneration. Recent studies have found that transplantation of ECM-containing matrices increased Schwann cell attachment and viability. For example, when Schwann cells were added to an alginate matrix containing fibronectin, and subsequently transplanted into a rodent peripheral nerve lesion, conduits containing fibronectin displayed increased Schwann cell viability, leading to greater nerve regeneration. It should be noted that nerve regeneration was promoted due to the presence of Schwann cells, as well as the proregenerative effect of fibronectin (Mosahebi et al., 2003). Fibronectin and laminin also have a neuroprotective effect on Schwann cells. When these cells were cultured on fibronectin–laminin-coated surfaces, viability and cell attachment was greatly increased when exposed to apoptotic conditions as compared to when these cells were cultured on non-ECM coated surfaces (Mosahebi et al., 2003). These studies further suggest the utility of incorporating ECM matrices for increased proliferation and viability of neural and/or glial cells.

6.4.2.6 Preformed living scaffolds

In the last two decades, extensive progress has been made in the field of neural tissue engineering. Directed axon growth and cell migration along pathways formed by other cells is seen in nervous system development. It is considered crucial for proper formation of the nervous system, which includes appropriate axonal connectivity and localization of cellular constituents. Developmental mechanisms of neural growth and axonal pathfinding are now being fully utilized in tissue engineered living scaffolds, which often integrate the use of ECM components with living cells to provide contact guidance and secrete proregenerative factors. Cells are grown and utilized in creating constructs with specific geometrical, mechanical, and biological cues to allow for targeted neural tissue regeneration across 3D space. For example, East et al. developed 3D collagen gels containing aligned astrocytes. Astrocytes were first aligned within a tethered collagen gel, after which plastic compression was used to create stable collagenous sheets containing the aligned cells (East et al., 2010). The sheets were then rolled to create cylindrical constructs, and seeded with dissociated DRG neurons to test their ability to promote neuronal growth. It was found that neurites preferentially grew along the aligned astrocytes, and that their growth was enhanced in comparison

to control tubes (East et al., 2010). Similarly, another group created 3D nanofiber scaffolds containing aligned astrocytes. Here, scaffolds were created by placing electrospun poly-L-D-lactic acid nanofibers attached to an acetate frame on top of a collagen I hydrogel base. Glial cells were subsequently seeded onto the mesh. This process caused the astrocytes to align and proliferate upon the nanofibers (Weightman et al., 2013). Both of these approaches to create scaffolds of aligned astrocytes were engineered for repair of CNS injury and represent promising approaches that may subsequently support neuronal regrowth and regeneration. An alternative approach to creating constructs containing aligned glial cells is to create constructs containing long, aligned axonal tracts. These constructs are designed to utilize axon-mediated axonal outgrowth to support neural repair. This mechanism involves the growth of regenerating axons along preformed (ie, tissue engineered) axonal pathways, mimicking axonal growth along pioneer axons during nervous system development. In one application, a micrometer-scale tubular construct consisting of an inner ECM core comprised of a collagen and/or laminin matrix and an outer hydrogel shell, with primary neurons seeded on either end of the constructs was developed, and axonal projections were allowed to grow across the tubular constructs (Cullen et al., 2012). These micro-tissue engineered neural networks (micro-TENNs) exhibited robust neuronal survival and axonal extension in vitro, recapitulating the systems-level neuroanatomy of the brain: discrete neuronal populations spanned by long axonal tracts that may be used for targeted reconstruction of neural circuitry lost due to injury or disease in the CNS (Harris et al., 2016).

In contrast, for the PNS, much larger constructs were developed containing long, integrated, axonal tracts for peripheral nerve repair. To generate the tissue engineered nerve grafts (TENGs), two neuronal populations were plated on either side of an interface, once axonal networks form between the two populations; they were slowly separated in micrometer-scale increments using a custom mechano-bioreactor. These integrated axons respond to the forces by increasing in length as well as diameter, and this process was seen to encourage fasciculation (Pfister et al., 2004, 2006). To date, stretch-grown axonal constructs have been generated at lengths of 5–10 cm in 14–21 days, with even longer lengths likely attainable (Pfister et al., 2004, 2006). The TENGs were then encapsulated in a collagen matrix in order to preserve the structure of the axons as well as provide a biologically active matrix to promote cell/axon survival, and transplanted into rodent and swine peripheral nerve injury models to test its efficacy to promote regeneration. Although there are significant challenges to implementation, this approach is extremely promising for neuroregenerative medicine and may ultimately facilitate functional recovery for a number of currently intractable neurodegenerative diseases and neurotrauma.

6.4.2.7 Fabrication of scaffolds containing electrospun nanofibers

Several techniques have been implemented to fabricate fibrillar scaffolds for neuroregeneration due to the inherent properties of nervous system tissue, specifically the collagen fibers that are abundant in the ECM and provide structural support.

Popular techniques used to develop nanofibers include, but are not limited to, injection molding, thermally induced phase separation (TIPS), and electrodeposition. However, a promising and relatively simple method of fabricating nanofibers is electrospinning. Here, an electric charge is used to draw liquid from a polymer in the form of a fiber with a micrometer-scale diameter (Ziabacki, 1976). Recently, electrospinning has been employed to develop nanofibers that recreate the structure, diameter, and tensile strength of neural ECM. For example, nanofibers exhibiting physical and mechanical properties similar to neural ECM were created by electrospinning poly(L-lactic acid)-co-poly(ε-caprolactone) (PLCL) mixed with collagen type I and type III. The efficacy of nanofibers consisting of varying ratios of PLCL to either collagen I, collagen III, or both collagen I and collagen III was tested. Investigators found an increase in proliferation of mouse cerebellum stem cells seeded on the collagen blended nanofibers in comparison to cells seeded on PLCL-only nanofibers (Kijenska et al., 2012). PLCL nanofibers consisting of either blended laminin (PLCL-lam(B)) or PLCL-laminin core shell (PLCL-lam(CS)) nanofibers were also developed, exhibiting physiologically relevant fiber diameter and tensile strength. Interestingly, an increase in Schwann cell proliferation on core-shell nanofibers was seen. This was likely due to controlled release of the laminin from the core, which allowed for optimal levels of laminin for Schwann cell proliferation. Laminin core-shell nanofibers may also prove beneficial if mixing bioactive molecules, such as NGF, is desired. Due to the hydrophilic properties of these molecules, they are only soluble in aqueous solutions, and mixing these directly with the polymer in the organic solvents, as is necessary for many applications, may lead to denaturation of the proteins. The core-shell design of these nanofibers may protect against denaturation as well as allow for controlled release of the molecule (Kijenska et al., 2014). Similarly, another study was conducted wherein gelatin was blended into PCL nanofibers to mimic the fibrillar structure of neural ECM. Here, varying weight ratios of PCL to gelatin were tested for their effect on regeneration. It was found that blending PCL with gelatin promoted neurite extension when compared to PCL nanofibers alone (Ghasemi-Mobarakeh et al., 2008). Finally, the use of Schwann cells seeded on electrospun HA-gel nanofibers for peripheral nerve regeneration was explored. Again, various weight ratios of HA to gelatin were tested. Schwann cell attachment and proliferation did not seem to vary significantly when seeded on the different nanofibers. However, protein expression as well as F-actin alignment was increased when grown on nanofibers consisting of HA. Therefore, addition of HA into matrices for nerve repair may prove beneficial (Liou et al., 2013).

6.5 Conclusions

The ECM plays a key role in neural development, migration, and maintenance. The sensitivity to injury and significant regenerative challenges of the mature nervous system have required novel treatments to be developed. These challenges range from minimizing bleeding to achieving regrowth after neurotrauma. The specific homeostasis needed in the nervous system to maintain function is complex, and alterations of that

state often have significant deleterious outcomes. Moreover, extreme challenges in functional restoration following neurodegeneration result from the minimal regenerative capacity in the mature CNS, thereby requiring various strategies to assist in neural repair. Fortunately, ECM constituents are extensively employed by the body to achieve proper growth during development or ensure homeostasis and proper function during maturation. Therefore, numerous researchers are advancing the use of ECM molecules in nervous system repair. Current applications of ECM-derived neural implants are macroscopic, focusing on connective tissue reconstitution and repair in the CNS, and nerve conduits or wraps in the PNS. However, there is a significant number of preclinical experimental strategies that have potential to affect nervous system regeneration at the microenvironmental and molecular levels. As these promising strategies translate from the bench top to the bedside, integrated ECM constructs may advance restorative and regenerative neurosurgery.

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