Chapter 6
Bioactive Nanomaterials for Neural Engineering

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6.1 Introduction

6.1.1 Nerve Regeneration and the Roles of Extracellular Matrix Elements

Nervous system is a highly complex interconnected network and higher organisms including humans have limited neural regeneration capacity. Neurodegenerative diseases result in significant cognitive, sensory, or motor impairments. Following an injury in the neural network, there is a balance between promotion and inhibition of regeneration and this balance is shifted to different directions in central nervous system (CNS) and peripheral nervous system (PNS). More regeneration capacity is observed in the PNS compared to the CNS. Although, several mechanisms play roles in the inhibitory and growth-promoting natures of the CNS and PNS, extracellular matrix (ECM) elements are key players in this process. ECM is a three-dimensional environment where the cells migrate, proliferate, and differentiate (Rutka et al. 1988; Pan et al. 1997). After a comprehensive investigation of the interactions between the ECM proteins and cell receptors, the ECM environment was found to regulate significant cellular processes such as survival, proliferation, differentiation, and migration (Yurchenco and Cheng 1994; Aszodi et al. 2006). Its components have major roles not only in neurogenesis during development of the nervous system but also in normal neural functioning during adulthood (Hubert et al. 2009).

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6.1.1.1 Peripheral Nervous System

In the PNS, neurons (axons) and Schwann cells are the major cellular elements. Endoneurium tissue is the connective tissue that surrounds individual axon-Schwann cell units, whereas perineurium covers a fascicle of axons. Perineurium also acts as a barrier against fluxes of ionic and macromolecular compounds between connective and vascular tissues and endoneurium (Siegel et al. 1999). Epineurium is the outermost connective tissue, which covers the entire nerve (Bunge et al. 1989). In the endoneurium, Schwann cells are abundant, whereas fibroblasts form 10 % of the cell population (Verheijen et al. 2003). Myelination after axonal regeneration has a central role for functional outcomes, and proper ECM formation has strong influence on this process (Bunge 1993).

Basal lamina of PNS contains laminin, fibronectin, entactin, and heparan sulfate proteoglycans (Bunge 1993) and collagens (Shellswell et al. 1979). Laminin is synthesized by the Schwann cells, and it is considered to have the leading bioactivity in terms of growth, adhesion, and migration of these cells (Milner et al. 1997). Laminin has also been shown to have a critical effect on myelination during peripheral nerve regeneration in culture systems (Tsiper and Yurchenco 2002). As another basal lamina element, collagen is the major ECM protein and it is produced mostly by the fibroblasts and Schwann cells in fibrillary and nonfibrillar forms (Koopmans et al. 2009). Fibrous types of collagens: collagen I, III, and V are found in all three ensheathing layers of peripheral nerve tissue. Collagen type-I and III are present in small diameters in the external face of Schwann cell basal lamina, whereas collagen type-V colocalizes with them in addition to enveloping myelinating Schwann cells in the basal lamina (Chernousov et al. 2006). Schwann cells also produce a more glycosylated and nonfibrillar type of collagen, collagen IV, which is a principle component of basal lamina. Collagen IV has a role in integrating laminin, perlecan, nidogen, and other ECM proteins into a supramolecular structure (Hudson et al. 1993) in the basal lamina surrounding Schwann cells, the perineurial cells, and endoneurial capillaries (Koopmans et al. 2009). Fibroblasts produce a fibrillary network of collagens and provide the framework required for Schwann cell ensheathment of regenerating axons (Eather et al. 1986). Fibronectin is another important ECM protein that has a very defined and specific expression pattern to guide neuronal outgrowth (Sheppard et al. 1991). Interaction of fibronectin with collagen, heparin, fibrin, and integrins via its specific domains results in cellular responses including cell adhesion, Schwann cell motility, and growth (Ahmed and Brown 1999). Chondroitin sulfate proteoglycans (CSPG) are also abundant in the Schwann cell ECM; however, they show inhibitory activity in contrast to other ECM elements in the PNS tissue (Braunewell et al. 1995).

Although complete recovery of PNS is not common, especially for large gaps, PNS injury environment is more permissive for regeneration compared to CNS. Nonneuronal cells respond to injury and start a key event called “Wallerian degeneration” (Waller 1850). This process initiates a series of events, which together help clearance of inhibitory myelin debris and promotion of axon regrowth (Griffin et al. 1995). Axon degeneration starts several days after the injury, leaving

the tissues denervated (Gilliatt and Hjorth 1972). When calcium starts to influx from the ECM and internal Ca^{2+} stores to the injured axon (Stirling and Stys 2010), calpain is activated, a protease, which functions in cytoskeletal degradation and axonal degeneration (Wang et al. 2004). Schwann cells and fibroblasts secrete tropic and tropic factors, and detached Schwann cells go through proliferation. The basal lamina remains and guides endoneurium toward the distal site (Fu and Gordon 1997). Schwann cells form Bands of Büngner with the help of fibrin cables, where fibroblasts and blood vessels can also use as a guiding surface (Williams et al. 1983). Fibrin is later replaced by collagens produced by fibroblasts and laminin secreted by Schwann cells. Regeneration fails when the initial fibrin cable cannot be formed due to a large gap (Yannas et al. 2007).

6.1.1.2 Central Nervous System

Apart from the neurons and glial cells, ECM constitutes 10–20 % volume of the CNS (Bignami et al. 1993). While specific pathfinding, migration, and differentiation of the cells are regulated by specific ECM proteins during CNS development (Bandtlow and Zimmermann 2000), ECM components play role in stabilization of the structure, regulation of the synaptic plasticity, and prevention of aberrant synaptic remodeling throughout the adulthood (Dityatev and Schachner 2003). The matrix forms a dense network of proteins and glycans, facilitating the organization of the cells as well as providing structural support to them (Lau et al. 2013). Basal lamina, perineuronal nets, and interstitial matrix form the ECM structurally. Basement membrane is the tissue that covers the entire pial surface of the CNS and it comprises of collagen, laminin, nidogen, fibronectin, dystroglycan, and perlecan. On the contrary, the matrix surrounding the neurons, perineuronal nets, have a network majorly made up of proteoglycans, tenascin R, and other proteins (Kwok et al. 2011), which conserve and maintain synaptic plasticity. Interstitial network is formed by proteoglycans, hyaluronan, tenascins, and other linking proteins (Rauch 2007). Moreover, collagen, elastin, laminin, and fibronectin also participate in the structure of the network, however in smaller amounts (Lau et al. 2013).

Following a damage to the CNS, a series of molecular and cellular events occur resulting in inhibition of regeneration process. Glial scar tissue formation is triggered by the entrance of non-CNS elements to the CNS. Although it leads to inhibition of regeneration, one important beneficial role of glial scar is to preserve the damaged tissue, repair the blood–brain barrier (BBB), and minimize cellular degeneration and inflammatory burden (Silver and Miller 2004; Bush et al. 1999). First, macrophages migrate to the injury site from the blood due to BBB disruption. Then, oligodendrocyte precursors migrate to the injury site in massive numbers. Finally, astrocytes proliferate and migrate to the area to fill in the injury area and become reactive, which is a process called “reactive astrogliosis” (Fawcett and Asher 1999). Reactive astrocytes produce glial fibrillary acidic protein (GFAP) after CNS injury, which can also be used as a marker for glial scar formation. Although GFAP production is similar to collagen fibers, they are important in regeneration process.

ECM of CNS is composed of protein or proteoglycan-based aggregates, whereas native PNS ECM has a fibrous structure (Alovskaya et al. 2007).

Besides producing growth promoting factors, astrocytes also produce four different types of proteoglycans, which are made up of a core protein and sulfated glycosaminoglycan chains attached to the sides that are inhibitory to regeneration: heparan sulfate proteoglycan (HSPG), dermatan sulfate proteoglycan (DSPG), keratan sulfate proteoglycan (KSPG), and CSPG (Larsen et al. 2003). Hyaluronic acid is another carbohydrate, which is also present in the ECM of CNS. It interacts with proteoglycans to form a mesh-like structure in the perineuronal network (Kwok et al. 2010). During development, CSPG plays a role in inhibitory patterning of neuronal pathway (Tang et al. 2003). In healthy adult perineuronal networks, they are involved in stabilization of synaptic plasticity (Hunanyan et al. 2010). However, upregulated levels of CSPGs are known to increase glial scar in the mature spinal cord and brain (Becker and Becker 2002), and they inhibit neurite outgrowth extensively in vitro (Sharma et al. 2012). They are upregulated within 24 h following injury and they remain at the injury site for months (McKeon et al. 1999; Jones et al. 2003). Mechanism of CSPGs inhibition is thought to be both nonspecific, through the contact of negatively charged glycosaminoglycan chains, and specific through signaling mechanisms by interacting with PTP and receptors (Dickendesher et al. 2012; Sharma et al. 2012).

6.1.2 Blood–Brain Barrier and Blood–Spinal Cord Barrier

BBB and blood–spinal cord barrier (BSCB) are mechanisms that act as shields between CNS and blood and they preserve homeostasis in organisms with well-developed CNS (Abbott 2005). Even though they have similar morphological characteristics and functions such as preservation of CNS, BBB and BSCB are considered to be different processes. Both BBB and BSCB are composed of nonfenestrated endothelial cells, basement membrane, pericytes, and astrocytic end processes. Endothelial cells have tight junctions via claudin, occludin, and adherens junction molecules (Abbott et al. 2006). BSCB differs from BBB in terms of permeability of different molecules. Furthermore, there are glycogen deposits in the microvessels, which are not present on the cerebral vascular structure (Sharma 2005) and are thought to serve as an endogenous energy source. Cellular components of BBB are microvascular endothelium, astrocytes, basement membrane, pericytes, and the neurons that are in physical proximity to the microvascular endothelial cells. The neurons in the brain and the spinal cord communicate through chemical signals via neurotransmitters and modulators, and electrical signals via synaptic potentials and action potentials, which form a complex network. Ionic movements across the neuronal membranes are involved in the signaling processes. There are also the ionic fluxes, which maintain the resting membrane potentials stable and the ionic movements involved with electrochemical signals are transmitted on this background of ionic fluxes. In order for signal transmission to be precise, reliable, and consistent,

ionic composition of the brain extracellular milieu needs to be preserved against the rapid fluctuations of ionic composition in the blood caused by physical exercise or food intake. There is evolutionary evidence that ionic movements were the major factor driving barriers between CNS and blood, and the barriers gained other functions subsequently (Abbott 1992).

Brain microvascular endothelial cells (BMVEC) are responsible for regulation of function such as transportation of micro and macronutrients, receptor-mediated signaling, regulation of osmotic pressure, and leukocyte trafficking. They impede free exchange of solutes (Ohtsuki and Terasaki 2007) with the exception of lipid-soluble molecules smaller than 400 Da with less than nine hydrogen bonds, which are able to cross BBB via lipid mediated diffusion (Pardridge 2007). Structurally, the cells are connected to each other via tight junctions (TJs), adherens junctions (AJs) (Hawkins and Davis 2005), and gap junctions (Boulay et al. 2015), which are required for the compact characteristics of the barrier. Their main role is to restrict passage of unwanted molecules between blood and brain by forming a continuous layer of membrane that does not contain fenestrae, which are normally found on the endothelial cells of blood vessels for rapid exchange of molecules. Along with the physical barrier created by junctional elements and low transcytotic activity, endothelial cells also create an enzymatic barrier against potential lipophilic substances, such as lipophilic drugs and toxic substances (El-Bacha and Minn 1999), which provides a metabolic barrier to the brain (El-Bacha and Minn 1999). Neurons at the periphery are connected through astrocytic interactions to the BBB (Abbott et al. 2006) and together with the other neovascular unit elements (astrocytes and pericytes), they provide required paracrine signals to the endothelium (Deane and Zlokovic 2007) and control BBB permeability, structure, and function (Abbott et al. 2006).

The CNS barriers protect nervous system homeostasis and they control molecular traffic, toxins, neuronal signaling, low protein environment in CNS, and neuronal circuits. They reduce cross talk by separation of central and peripheral neurotransmitter pools and ensuring minimal inflammatory response and functional impairment during immune surveillance (Abbott 2013).

6.1.3 Challenges in Engineering Biomaterials for Nervous System Repair

Following a nervous system injury, regeneration capacity usually depends on the extent of the injury, the distance of the injury to the cell body, and biological status of the patient (morbidity, age, etc.) (Faroni et al. 2015). The PNS and CNS respond to injury in their own unique way. In the PNS, Wallerian degeneration occurs in the distal end following a series of pathophysiological events. The distal portion of the nerve is degenerated and the cellular debris is digested by the macrophages and monocytes (Stoll et al. 1989). Schwann cells form the Bands of Büngner in order to guide regenerating axonal sprouts to its synaptic target (Chaudhry et al. 1992;

Schmidt and Leach 2003). During the extension process, bridging the gap between the two ends and optimizing the environment physically, chemically, and biologically is a strategy that has been followed (Schmidt and Leach 2003). In PNS, the challenge is to find a perfect alternative to autologous nerve grafts: eliminating risks of secondary surgeries and precluding secondary damage on the body. Even though structural plasticity is achieved clinically, functional plasticity does not always reach complete state and it still is another principal consideration in PNS regeneration studies. Autologous nerve graft treatment shows 50 % clinical functional recovery (Lee and Wolfe 2000). Furthermore, use of natural proteins for therapeutic purposes can cause immunogenic reactions. Sustained delivery or storage of growth factors are also required in order for effective usage of growth factors (Schmidt and Leach 2003).

CNS has much smaller capacity to regenerate; thus, CNS therapies are more challenging. Embryonic spinal cord and peripheral nerve grafts have been shown to support regeneration of CNS fibers, however failed to successfully grow through the CNS–PNS transition zone (Bernstein and Goldberg 1995) (Carlstedt 1997). CNS does not have a permissive nature for regeneration. There are many reasons behind the obstructive environment of CNS injuries. Regeneration-associated genes are expressed at low levels in the CNS (Bulsara et al. 2002). Following the CNS injury, glial scar is formed and inhibitory molecules are released at the site of injury. Cellular debris and inhibitory myelin components are cleared much slowly compared to the PNS as a result of low infiltration levels of macrophages through the brain–spinal cord barrier (Avellino et al. 1995). Moreover, astrocytes proliferate at the site of injury, in a similar way to Schwann cell proliferation, however, in contrast, creating an inhibitory environment and becoming reactive astrocytes (McKeon et al. 1991). Thus, nerve regeneration studies focus on suppressing the inhibitory nature of the nervous system injuries and future directions in PNS and CNS repair include combining multiple cues at a time to increase the regeneration capacity (Schmidt and Leach 2003). BBB is another obstacle for drug delivery to the brain, considering that intracranial injections are much more invasive than other administration (i.e., intravenous, oral) methods. Another challenge for drug delivery is accurate targeting of the correct population of the cells.

6.2 Biomaterial Design for Peripheral Nerve Repair

PNS injuries most commonly caused by trauma (Ichihara et al. 2008), bone fractures, or joint dislocations (Zumwalt and Wooldridge 2014). They result in partial loss of sense or motor function in the distal segment of the injured axon (Navarro et al. 2007). The potential to achieve functional recovery depends on the severity of the damage at the axon, nerve tube, or connective tissues at the injury site, timing of the surgery, surgical technique used, and postoperational rehabilitation (Lanaras et al. 2009; Barton et al. 2014).

As a clinical strategy, the two ends of the nerve are sutured if the gap between the distal and proximal end is <2 cm. However, other alternatives are considered in

cases of nerve segmental loss with a consequent gap longer than 2 cm because of the tension that emerges when two ends are sutured to each other (Johnson et al. 2005). Nerve grafting or nerve conduits are standard procedure in cases like these (Siemionow and Brzezicki 2009; Pabari et al. 2010). During autograft nerve transplants, a nerve segment is transplanted from another region of the same patient. Clinically, autografts are accepted as the "gold standard" because of being nonimmunogenic and having best possible combination of the natural environment required for nerve regeneration. Autografts provide bridging of two ends, allowing physical adherence guidance and proper support for Schwann cell proliferation. On the other hand, they have some drawbacks such as sensation at the donor site, creating a second incision in the body, and having a limited supply of the donor site. Cadaveric nerve allografts are another option as nerve grafts, which do not require a second incision on the patient, however, compel systemic immunosuppression (Trumble and Shon 2000; Pollard et al. 1971; Mackinnon et al. 1982; Lassner et al. 1989; Gulati and Cole 1990; Gulati 1998). This technique is usually preferred in cases like severely damaged segmental nerve loss (Ray and Mackinnon 2010). Although nerve grafts seem plausible because of their optimal nature for regeneration, only 50 % of patients with autograft nerve transplants regain functional nerve regeneration (Lee and Wolfe 2000) and the drawbacks of these techniques have led to development of synthetic and biological nerve guidance conduits (Fansa et al. 2001; Walsh et al. 2009; Glasby et al. 1986). For this purpose, a primary concern should be mimicking the native environment of the PNS for optimal nerve regeneration. Some of the important points that should be taken into consideration while designing a peripheral construct are supporting axonal migration, promotion of viability, and proliferation of Schwann cells; proper storage of growth factors; and providing multiple cues from the native ECM (Evans 2000). In this regard, biodegradable hollow neural guidance channels can be used with ECM mimicking matrix fillers, coatings, and growth factor storing scaffolds. Schwann cell transplantation is another alternative that can be delivered within these scaffolds (De Luca et al. 2014).

6.2.1 Engineering Topographical and Mechanical Properties for Neural Guidance

Biocompatible and bioactive materials have been utilized in order to mimic physical, chemical, and biological properties of the native neural tissue. Cells are distinctly responsive to every cue in their environment including surface topography, stiffness, and interacting fiber diameter and change their behavior accordingly (Georges and Janmey 2005; Pedersen and Swartz 2005; Khatiwala et al. 2006; Curtis and Riehle 2001). Grooves, micro- and nanofibers, gels, and films have been studied in order to promote and direct neuronal outgrowth and enhance neuronal attachment (Xie et al. 2010; Sun et al. 2010; Mobasseri et al. 2013; Daud et al. 2012; Bell and Haycock 2012). Instead of using hollow guidance tubes that lacks physical

properties of the native nerve structure, studies have focused on developing materials that guide the axons to the distal site of the injury. Lumen filling materials are used for this purpose to provide contact, attachment, and growth of the cells (Chen et al. 2006; Jiang et al. 2010). Naturally, Schwann cell basal lamina is a favorable environment for physical guidance. It consists primarily of laminin and collagen that represent aligned, nanoscale features (Bunge and Bunge 1983). Neurite outgrowth of chick dorsal root ganglia (DRG) neurons was intensely improved on aligned nanofibrous surfaces, which demonstrates the importance of these features (Kim et al. 2008; Corey et al. 2007). Incorporating Schwann cells is also a strategy; aligned collagen poly-E-caprolactone (PCL) filament constructs seeded with Schwann cells have shown that DRG cells had enhanced and oriented neurite outgrowth in vitro (Ribeiro-Resende et al. 2009).

Along with these factors, porosity of the conduit is also important in axonal regeneration. Pores of the conduits enable inward diffusion of ECM proteins and growth factors (Kim et al. 1993), and outward diffusion of waste products. In addition to this, infiltration of connective fibrous tissue should also be prevented (Wang et al. 2009) and regeneration was observed to proceed into microsized pores (Oh et al. 2013). Therefore, the pores of the conduits should be wide enough for growth factor and waste product diffusion and narrow enough to prevent fibrous tissue infiltration and regeneration toward the pore. "Roll and seal" model aligned nanofibrous conduit-derived pores have been shown to trigger greater neurite outgrowth and functional recovery compared to aligned microfibrous conduit-derived pores (Jiang et al. 2014).

Nervous tissue is a soft tissue and is sensitive to the mechanical stiffness of the environment. Natural stiffness of the peripheral neural tissue has stiffness value between 150 and 300 kPa, whereas glial cells and neurons individually have stiffness values ranging from 0.5 to 1.6 kPa (Jalili-Firoozinezhad et al. 2014). Therefore, resemblance of stiffness to the native environment is an important factor that plays role on nerve regeneration. Agarose gel stiffness (density) was showed to have an inversely proportional relationship with neurite extension rate of DRG cultures (Balgude et al. 2001). PEG-based hydrogels have also been studied for nerve regeneration purposes and as the stiffness of the PEG-based hydrogel increased, PC12 cells showed reduced neurite extension. In addition, below a threshold value of stiffness, neurite outgrowth of PC12 cells decreased drastically (Leach et al. 2007), which proves that neurons require a defined range of intermediate stiffness (Hoffman-Kim et al. 2010). These studies indicate the significance of mechanical properties of native nerve tissue in designing biomaterials for peripheral nerve repair.

6.2.2 Surface Chemistry and Biochemical Modifications to Increase Nerve Regeneration

Nerve guidance conduits require some additional properties such as surface modification and some biochemical cues in order to promote axon guidance, Schwann cell proliferation, adhesion, and migration (Gu et al. 2014). These modifications may be

in the form of protein coatings, chemical/physical treatment of the surface, or protein mimetic peptides presented on the biomaterials (Chung and Park 2007). In order to create the native environment of the healthy nerve tissue, ECM proteins are considered to have great potential for functionalization of the conduit surface. Collagen, fibronectin, and laminin are examples of some major components of the ECM that have been used for this purpose (Yu and Bellamkonda 2003; Armstrong et al. 2007; Koh et al. 2010). Laminin, in particular, has been used most frequently for surface modification or ECM mimicking purposes among others due to its ability to improve neurite extension and provide Schwann cell adhesion, proliferation, and migration (Yu and Bellamkonda 2003; Silva et al. 2004; Yu et al. 1999; Itoh et al. 2001; Rangappa et al. 2000; Rutkowski et al. 2004; Toba et al. 2001; Matsumoto et al. 2000; Koh et al. 2010; Bellamkonda et al. 1995). Collagen and fibronectin also have regenerative capacity in terms of Schwann cell adhesion, proliferation, and neurite outgrowth improvement; however, outcomes have shown to be significantly lower than that of laminin in terms of regeneration (Yu and Bellamkonda 2003; Armstrong et al. 2007; Koh et al. 2010). Despite the major impact of ECM protein-based functionalization of the materials on neural regeneration, they are difficult to synthesize due to their large size (~900 kDa) (Santiago et al. 2009; Itoh et al. 2001). An alternative to using large ECM protein modifications is protein mimetic short peptide sequences, which are more stable, less immunogenic, and relatively low molecular weight. These short peptides are usually designed to be recognized by the cellular receptors and they are represented on a surface. Due to their small size, they have a high surface density; thus, there is more interaction for signaling events and cell attachment (Itoh et al. 2001; Chung and Park 2007).

A widely used short peptide sequence is RGD (Arg–Gly–Asp), which is an integrin binding amino acid sequence found in fibronectin, laminin, and other ECM molecules and has been used for inducing cell attachment. IKVAV (Ile–Lys–Val–Ala–Val) (Tashiro et al. 1989) and YIGSR (Tyr–Ile–Gly–Ser–Arg) are found at the laminin b chain and RNIAEIIKDI (Arg–Asn–Ile–Ala–Glu–Ile–Ile–Lys–Asp–Ile) belongs to laminin g chain, which all mimic laminin. HAV (His–Ala–Val) sequence mimics N-cadherin, which is an adhesive and regulatory protein found on both neurons and glial cells (Chung and Park 2007; Itoh et al. 2003; Santiago et al. 2009; Itoh et al. 2001). Functionality of these peptides has been assessed in various applications. Adams et al. showed that DRG neurons, that were grown on gradients of photoimmobilized IKVAV bound polystyrene grids, preferentially directed their neurites toward higher concentration of IKVAV containing surface (Adams et al. 2005). In another study, melt coextruded aligned poly(ε-caprolactone) (PCL) fibers were modified with photochemical gradient of IKVAV peptide, which provided directional cues for neuronal outgrowth of PC-12 cells (Kim et al. 2015).

Schense et al. (2000) evaluated the effects of five ECM mimetic peptides: RGD, IKVAV, YIGSR, RNIAEIIKDI, and HAV within a fibrin matrix. In this study, each peptide-coated matrix showed increased neurite extension than uncoated fibrin matrix in vitro. Moreover, synergistic effect of the four laminin mimetic peptides showed significant increase in terms of neurite outgrowth compared with the single peptide-coated matrices (Schense et al. 2000). In the in vivo studies of the same group, they tested same materials as neural guidance channel fillings for their

regenerative capacity in dorsal root ganglion models. Synergistic effects of four laminin mimetic peptides also showed similar effects to the in vitro studies (Schense et al. 2000). Similarly, PCL scaffolds with RGD peptide functionalization resulted in enhanced Schwann cell adhesion as well as axonal interaction in vivo (Santiago et al. 2009).

Another method to produce nanofibrous scaffolds, while controlling diameter, porosity, and surface morphology, is electrospinning (Pham et al. 2006; Subbiah et al. 2005). A variety of polymers can be electrospun on aluminum surfaces and these polymers are also known to contribute to neural regeneration by their nanofibrous topography. In addition, their surface can be functionalized by bioactive epitopes or chemical groups (Pham et al. 2006; Prabhakaran et al. 2008). Bellamkonda et al. showed that multiple layers of aligned acrylonitrile-methacrylate (PAN-MA) nanofibers stacked within semipermeable nerve guidance tubes showed highly aligned and enhanced neurite extension of DRG cultures and in vivo PNS regeneration studies (Clements et al. 2013). In another study conducted by Ahmed et al., electrospun nanofibers were biofunctionalized by tenascin-C-derived peptides, which increased cell adhesion compared to poly-L-lysine-coated glass surfaces (Ahmed et al. 2006).

Self-assembled peptide amphiphile (PA) nanofibers are used as matrices similar to ECM characteristics. With these nanostructures, the neural microenvironment can be manipulated in a way that ECM mimicking peptides are represented on the surfaces of the nanofibers. These peptide nanofibers are promising materials due to their nonimmunogenic, biodegradable, and bioactive nature (Tan et al. 2012). These materials consist of a hydrophobic alkyl tail, β-sheet forming amino acids, and a hydrophilic bioactive epitope. In aqueous solutions, oppositely charged PA molecules self-assemble into nanofibers and form gels (Cui et al. 2010). Cooperative effect of laminin mimetic IKVAV PAs and heparan sulfate proteoglycan-derived PAs promoted neurite outgrowth of PC-12 cells significantly. Besides, the inhibitory environment caused by chondroitin sulfate was also overcome by these materials (Mammadov et al. 2012). The protein or peptide modification is a significant approach for neural guidance conduit functionalization.

6.2.3 Enhancing Regeneration by Electrical Stimulation via Conductive Biomaterials

Electrical stimulation is a method to accelerate nerve regeneration (Seil and Webster 2010). Since neurons are electroactive cells, they respond to electrical stimulation by neurite extension and differentiation. Both direct and alternating current (DC and AC) within a voltage range is known to promote neurite outgrowth. DC was shown to enhance increased and directed neurite outgrowth (Borgens et al. 1979). One mechanism of promotion of neurite outgrowth by electrical stimulation is by upregulation of growth-associated genes. For instance, cyclic adenosine monophosphate

(cAMP) production was upregulated upon electrical stimulation of DRG cells (Udina et al. 2008). Polyaniline, polypyrrole, polythiophene, and polyacetylene are some known conductive substrates (Marquardt and Sakiyama-Elbert 2013; Schmidt et al. 1997). Poly (D,L-lactide-co-epsilon-caprolactone) (PDLLA/CL) nerve guidance (NGCs) fabricated with polypyrrole enhanced neurite outgrowth compared to PDLLA/CL conduits alone (Zhang et al. 2007). In another study, high-voltage electrical stimulation of PLGA films significantly increased number of total neurites and myelinated axons (Bryan et al. 2004).

Polypyrrole and polyaniline both have excellent conductive and antioxidant properties in terms of cellular stimulation; however, their nonbiodegradable structure limits their usage in nerve regeneration studies (Gu et al. 2014). As an alternative strategy, blending of these materials with other biodegradable biomaterials has been proposed (Ghasemi-Mobarakeh et al. 2011). Rivers et al. were successful in synthesizing a conductive polymer by binding pyrrole oligomers to thiophene via ester linkages, so that ester linkages get cleaved by esterases in vivo (Rivers et al. 2002). In another study, a block copolymer of polyglycolide and aniline pentamer showed electroactivity and degradability (Ding et al. 2007). Several studies show the effect of electrical stimulation on the axis of neural cell division, neuronal polarity, and directed neurite outgrowth (Nguyen et al. 2013; McCaig et al. 2005; Yao et al. 2011). All these studies prove that electrical stimulation has a noteworthy effect on axonal regrowth acceleration.

6.3 Biomaterial Design for Central Nervous System Repair

Any damage to CNS can be destructive due to loss of communication between healthy neurons, and it can cause neuronal degeneration and eventually cell death. Due to the low regeneration capacity of CNS, people with CNS trauma or neurodegenerative disorders suffer from lifelong consequences and there is a significant demand for new strategies to overcome the progressive cell death as well as to induce tissue regeneration. The failure of neurons in the CNS to communicate with each other after injury is mostly due to the lack of supporting environment for regeneration around the damaged neurons rather than characteristics of the cells (Richardson et al. 1980). For regeneration process of CNS neurons, first the survival of injured neuron is required, so that it can connect with its target. However, making contact is not enough for functional recovery; remyelination of the axons and properly functioning synapses on the target neurons are also required. The strategies to generate a biomaterial for central nerve repair should focus on removal of inhibitory environment, axon guidance, manipulation of cell signaling, increasing the local concentration of neurotrophic factors and suitable drugs, and providing artificial microenvironment to fill the gap occurred as a result of injury (Horner and Gage 2000).

6.3.1 *Mimicking Extracellular Matrix of Central Nervous System*

ECM is the surrounding environment of cells composed of proteins such as laminin and fibronectin, glycosaminoglycans, proteoglycans, and different types of soluble factors (Zimmermann and Dours-Zimmermann 2008). It provides structural, biological, and chemical support to the cells through modulating cell adhesion, proliferation, migration, cell-to-cell interaction, and differentiation. Within the scope of tissue engineering, focusing on the development of in vitro cell culture environments, which mimic the natural ECM of specific cell types has received a lot of attention (Holmes 2002; Lutolf and Hubbell 2005). For nervous system applications, synthetic materials are especially attractive, because their chemical, mechanical, and physical properties can be specifically modified to mimic a particular area within the nervous system (Schmidt and Leach 2003).

6.3.1.1 Chemical Signals

ECM proteins regulate cell fate including proliferation, migration, and differentiation through interacting with cell surface receptors. One of the major differences of CNS from other systems is the composition of ECM proteins. Many ECM proteins, including collagen and fibronectin, are abundant in other tissues, whereas there is almost none in CNS. On the other hand, there are different types of proteoglycans present between neurons and glial cells (Li et al. 2012).

While designing a biomaterial, the chemical signals provided by these specific proteins can be introduced into the system by incorporating the bioactive sequence for specific interaction depending on the nature of tissue of interest. For instance, arginine–glycine–aspartate (RGD) peptide sequence derived from fibronectin was found to bind to integrin proteins and function in cell adhesion (Pierschbacher and Ruoslahti 1984; Prowse et al. 2011). Later, several ECM-derived short sequences have been identified and used for the interaction with integrin proteins to take benefit of cell–ECM interactions. Due to the important role of laminin protein in ECM of nervous system, laminin-derived short sequences were identified. Tyrosine–isoleucine–glycine–serine–arginine (YIGSR) peptide sequence was used to promote cell adhesion in in vitro studies (Graf et al. 1987). Another sequence derived from laminin, isoleucine–lysine–valine–alanine–valine (IKVAV), was also discovered and found to promote neurite outgrowth (Tashiro et al. 1989). After the discovery of these small peptide sequences, they were included in nanomaterial surfaces with different forms and used in both in vitro and in vivo studies. These peptides, rather than whole proteins, have become more favorable for tissue engineering studies, since they are more stable and easy to synthesize. While fibronectin-derived synthetic peptide (GRGDS) was used in gellan gum hydrogels to enhance the cell adhesion of neural stem/progenitor cells in in vitro studies (Silva et al. 2012), laminin-derived IKVAV sequence was used in self-assembled peptide nanofibers as

a therapeutic system in a mouse model of spinal cord injury (SCI) (Tysseling-Mattiace et al. 2008).

Many soluble factors have important roles in NSC differentiation into specific lineages. Therefore, they can be incorporated within hydrogels to induce neural differentiation. For instance, through incorporation of neurotrophin-3 (NT-3) into chitosan hydrogels, NSC differentiation toward neurons was achieved (Li et al. 2009). On the other hand, when FGF-2 was incorporated into PEG hydrogels, NSCs preferred to stay at undifferentiated state in spite of the addition of differentiation medium (Freudenberg et al. 2009).

Biomaterial studies have also focused on neurotransmitters, which are important chemicals for transfer of messages between the cells of the brain. Acetylcholine-like biomimetic polymers, including both a bioactive unit (acetylcholine-like unit) and a bioinert unit (PEG unit), were studied with primary hippocampal neurons. These polymers have potential therapeutic use in neural tissue applications related with neurotransmitter-related diseases such as Alzheimer's disease through modulating the growth of hippocampal neurons (Tu et al. 2011).

6.3.1.2 Mechanical and Physical Cues

Besides the chemical signals to induce neural regeneration, mechanical and physical properties of the biomaterial system, such as stiffness and dimensionality, have to be taken into consideration to mimic the natural environment of nervous system. Mechanical properties of the material could contribute to differentiation into different lineages and sometimes, elasticity of the material can override the effect of chemical signals as shown in mesenchymal stem cells, which did not display any response to osteogenic growth factors when plated on soft surfaces (Engler et al. 2006).

The mechanical properties of the substrate show highly selective and specific effects in regenerative studies of central nervous system. Although brain and spinal cord are the softest tissues in human body with elastic moduli around 2 kPa, when glial scar occurs as a consequence of an injury, stiffness of this local area can become higher which forms both physical and chemical obstacles to neurite extension and regeneration in nervous tissue injuries. Soft materials have become more favorable in the studies of CNS tissue due to the selective response of neurons and astrocytes to matrix stiffness (Georges et al. 2006). In addition, gels with low elastic moduli were found to selectively induce neuronal development. It was previously shown that rat adult NSCs primarily differentiated into glial cells when cultured on stiff substrates having elastic moduli between 1 and 10 kPa, whereas soft materials primarily gave rise to neurons. Also, the highest amount of neurons was obtained when culturing on interpenetrating polymer network hydrogel with elastic modulus of 0.5 kPa, which is close to physiological mechanical properties of brain tissue (Saha et al. 2008). While spinal cord and cortical brain neurons favor soft materials to extend their neurites (Balgude et al. 2001; Saha et al. 2008), astrocytes generate stress fibers and they are more activated on the surfaces with high elastic modulus (Georges et al. 2006).

Another important factor about the physical structure of the material is the dimensionality of the substrate. Although two-dimensional (2D) cell culture studies are more commonly preferred because environmental control, cell observation, and manipulation are easy, three-dimensional (3D) studies have great importance as they provide a more realistic model for in vivo studies. In one study, hippocampal neurons were encapsulated into 3D aragonite matrix and compared to ones seeded on 2D surface. The cells showed higher survival rate in 3D cell culture compared to 2D conditions (Peretz et al. 2007). Also, Cunha et al. decorated the 3D biomaterial scaffold with RGD, BMHP1 (bone marrow homing peptide 1), and BMHP2 motifs, for adult NSC culture (Cunha et al. 2011). They provided deeper understanding about the cell behavior in 3D scaffolds which is required for future clinical applications. 3D gel matrices were also studied in injured brain model. Laminin-derived IKVAV motif was linked to self-assembling peptide RADA (16) to form a functional 3D peptide-based scaffold for NSC encapsulation. Beside differentiation of NSCs into neural cell in in vitro conditions, injection of this hydrogel into damaged brain tissue to fill the cavity and form a bridge for the gap eventually led to improvement in brain tissue regeneration (Cheng et al. 2013).

6.3.2 Approaches for Drug Delivery to Central Nervous System

The complexity of the nervous system is an important criterion that should be taken into consideration while designing a system for drug delivery. Biocompatible materials for drug delivery are desired to promote neural regeneration through releasing the cargo in a controlled manner while maintaining the integrity of healthy tissue. However, the presence of BBB and BSCB is the primary problem to deliver drug to CNS and limit the efficacy of drug delivery of therapeutics through forming anatomical, transport, and metabolic barriers. Therefore, different strategies should be considered to enhance drug delivery to CNS, including material properties, drug selection, and delivery method.

6.3.2.1 Material Properties and Methods for Drug Delivery

While designing new materials for neural tissue engineering, it is important to choose appropriate materials for nervous system. The chemical and physical properties of the material must be well evaluated in order to provide controlled release through degradation rate with suitable dimensions for the injected site. Depending on the purpose, you can use different synthetic materials with different mechanical properties and release profile so that the immune response can be modified through altering the composition of the material. For instance, Poly (ethylene glycol) (PEG) has the property to resist cell adhesion and protein adsorption (Alcantar et al. 2000), which contribute to minimize immune response. Further modifications with bioactive epitopes for cell adhesion or mimicking ECM can be used to provide cell

migration into the scaffold, which also contributes to regeneration (Benoit and Anseth 2005; Groll et al. 2005).

Poly (ethylene-covinyl acetate) (pEVA) is another commonly used delivery system used in neural tissue engineering studies since it is a nondegradable and biocompatible scaffold, which makes it a favorable choice. Stability is another important property of pEVA for drug delivery over an extended period. It has been used in nerve growth factor (NGF)- and NT-3-releasing guidance channels to induce regeneration in transected rat dorsal root (Bloch et al. 2001).

To overcome the BBB penetration problem, many different carrier systems have been developed and used in neural tissue engineering studies. The techniques used as delivery system can be classified as systemic and local delivery.

Systemic drug delivery is performed through intravenous or intraperipheral injection, but it requires high dosages to fulfill the therapeutic effect and can influence nontarget tissues due to systemic toxicity. Therefore, while designing systemic drug carriers, it is important to provide properties resisting to long circulation and favorable surface properties for endothelial cell interaction (Misra et al. 2003). For systemic drug delivery, liposomes and polymeric NPs have been extensively studied for brain drug delivery (Garcia-Garcia et al. 2005). Liposomes are biocompatible and biodegradable delivery systems, and their surfaces are generally modified with hydrophilic polymers to deal with plasma clearance of liposomes (Lian and Ho 2001). Generally, poly(ethylene glycol) (PEG) is used for an additional layer, which increases blood circulation time of liposome (Garcia-Garcia et al. 2005). For penetration of liposomes through BBB, the carrier system can be developed by active targeting, which modifies the distribution of liposomes through an antibody or a ligand conjugation that is eventually recognized by the receptor specific to target tissue (Schnyder and Huwyler 2005). Therefore, by combining the effect of PEG on extended circulation time and specificity due to an antibody or a ligand conjugation, delivery can be obtained through the BBB. Liposomes have been used for the treatment of CNS diseases including brain tumors, infection, and ischemia (Zhong and Bellamkonda 2008).

Polymeric nanoparticles (NPs) ranging from 10 to 1000 nm in size can be used as carrier polymers encapsulated or covalently attached to therapeutic drugs (Lockman et al. 2002). Polymeric NPs are more stable against the biological fluids when compared to liposomes. Moreover, their structures are more suitable for controlled and sustained drug release over a period of time after injection. Generally, poly(alkylcyanoacrylates) (PACAs), polyacetates, polysaccharides, and copolymers are used for NP synthesis (Garcia-Garcia et al. 2005). For instance, drugs including dalargin, loperamide, tubocurarine, and doxorubicin have been delivered to the CNS by Polybutylcyanoacrylate (PBCA) NPs (Zhong and Bellamkonda 2008). NPs can also be coated with hydrophilic polymers, such as PEG, to increase their uptake (Brigger et al. 2002).

To manage systemic toxicity of the drugs and increase their effectiveness, local delivery systems are favorable. Local delivery of therapeutic drugs with a biocompatible carrier provides an advantageous method at the target region. This approach also bypasses the BBB penetration problem. For CNS, PLGA and poly-

anhydride poly [bis(p-carboxyphenoxy)] propane-sebacic acid (PCPP-SA) are most commonly used biodegradable polymers for local drug delivery. For instance, PLGA microspheres were used as local delivery for antitumor agents such as 5-fluorouracil and platelet factor 4 fragment to treat brain tumors (Benny et al. 2005; Menei et al. 1996). PLGA microspheres were also used for neurodegenerative diseases. Dopamine and noradrenaline delivery with PLGA microspheres was used as a therapeutic strategy for Parkinson's disease (McRae and Dahlstrom 1994). Also, PLGA microparticles were used as NGF carriers for the protection of neurons from excitotoxin-induced lesions (Benoit et al. 2000).

6.3.2.2 Drug Selection

Depending on the tissue type to be regenerated, a specific therapeutic drug or combination of different drugs can be selected. Within the potential therapeutic drugs for neural tissue engineering, neurotrophins are the most common growth factors used for neural regeneration. Neurotrophins are composed of NGF, brain-derived neurotrophic factor (BDNF), NT-3, and neurotrophin-4/5. For instance, NGF works both in PNS and CNS. It especially enhances survival of cholinergic neurons, which makes it attractive for therapeutic studies against neurodegenerative disorders such as Alzheimer's disease (Siegel and Chauhan 2000). However, while designing a delivery system, it is important to know the right place to inject and dose required, because NGF can cause unwanted sensory neural fiber sprouting which terminally can cause chronic pain (Romero et al. 2001). In addition to NGF, NT-3 functions in neurogenesis through promoting the differentiation of new neurons. Moreover, studies have shown that NT-3 can promote cell survival and neurite outgrowth in motor neurons after spinal cord injury (Bloch et al. 2001; Grill et al. 1997).

Anti-inflammatory drugs can also be used in drug delivery systems to suppress chronic inflammation and immune response caused by implantation. Among these anti-inflammatory drugs, dexamethasone is one of the most commonly used drugs for this purpose. Although it is generally used to treat inflammatory diseases including arthritis and multiple sclerosis, some studies revealed promising results in neural tissue applications (Kim and Martin 2006).

Another drug category used for delivery systems in CNS is chemotherapeutic agents. Since chemotherapeutic agents can also affect nontarget tissues and cause systemic toxicity, targeted delivery of these drugs is quite critical and important. Glioblastoma is one of the most aggressive cancer types with short survival rates. The chemotherapeutic drugs cannot access to the brain by traditional chemotherapy applications because of the presence of BBB. Therefore, therapeutic potential of these drugs can be modified with delivery systems. For example, doxorubicin, one of the most potent antitumor agents, has been attached to the surface of poly(butyl cyanoacrylate) nanoparticles coated with polysorbate 80 and this drug was successfully transported into the brain to treat brain tumors (Gulyaev et al. 1999).

6.4 Concluding Remarks

Nervous system is the most complex system in the body due to the complex interactions of the cells with each other. Also, due to poor regeneration capacity of nervous system, development of new strategies for repair and regeneration of this system is in high demand. Moreover, lack of clinically available successful therapies makes these therapeutic studies more attractive. Biomaterials have been widely studied up to now and they provide highly promising strategies in treatment for disorders of nervous system. They can be tailored at molecular level and their structural and biochemical properties can be tuned, which allows improvement of therapeutic methods according to the purpose of treatment. Looking ahead, a wide range of materials, such as polymers and synthetic self-assembled systems, have already been developed, but it is still essential to generate other biomaterials considering the nature and all requirements of the tissue including physical, chemical, and biological demands.

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