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Carbohydrate based Biomaterials for Neural Interfacing

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Abstract

Neuroprosthetic devices that record and modulate neural activities have demonstrated immense potential for bypassing or restoring lost neurological functions due to neural injuries and disorders. However, implantable electrical devices interfacing with brain tissue are susceptible to a series of inflammatory tissue responses along with mechanical or electrical failures which can affect device performance over time. Several biomaterial strategies have been implemented to improve device-tissue integration for high quality and stable performance. Ranging from developing smaller, softer, and more flexible electrode designs to introducing bioactive coatings and drug-eluting layers onto the electrode surface, such strategies have shown different degrees of success but not without limitations. With their hydrophilic properties and specific bioactivities, carbohydrates offer a potential solution for addressing some of the limitations of existing biomolecular approaches. In this review, we summarize the role of polysaccharides in the central nervous system, with a primary focus on glycoproteins and proteoglycans, to shed light on their untapped potential as biomaterials for neural implants. Utilization of glycosaminoglycans for neural interface and tissue regeneration applications are comprehensively reviewed to provide a current state of carbohydrate-based biomaterials for neural implants. Finally, we will discuss the challenges and opportunities of applying carbohydrate-based biomaterials for neural tissue interface.

Graphical Abstract

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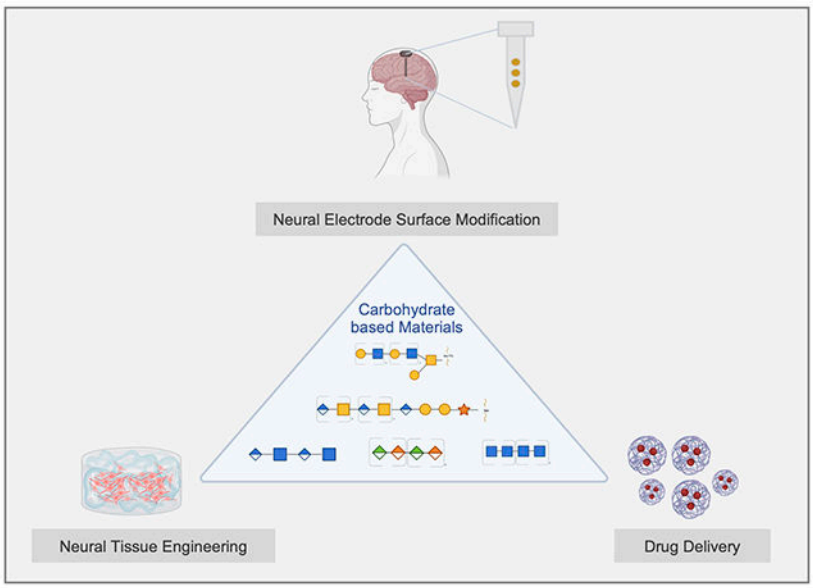

1. Introduction to Neural Interface Technology

1.A. Clinical and Research Significance

Neural interface devices are integral for stimulation of neural tissue and recording neural activities for neuro-modulatory and neuro-prosthetic applications in both central nervous system (CNS) and peripheral nervous systems (PNS). Existing clinically approved devices deliver current to neural tissues to modulate and restore lost functions. These devices include cochlear implants to restore hearing, retinal prostheses to restore vision, spinal cord stimulators for treating chronic pain, vagal nerve stimulators for treating epilepsy and depression, sacral nerve stimulators for bladder control, and deep brain stimulation (DBS) for relieving motor deficits related to Parkinson's and tremors[7,8]. Aside from clinically approved devices, brain-computer-interface (BCI) technologies are an active area of research, which detects brain activity using implanted neural electrode arrays, which is then decoded through classification algorithms[11], and subsequently used to control movement of an external machine such as prosthesis, wheelchair, or a robotic limb[14], or alternatively to directly stimulate and activate muscles[17,19] In addition to these clinically oriented devices, neural microelectrode arrays, have been critical in facilitating neuroscience breakthroughs for visual neurophysiology[20], discovery of place cells[21] and grid cells[22] to name a few[23].

1.B. Types of Neural Interface Devices

The wide variety of neural implants can be generally categorized based on their intended anatomical location (central nervous system (CNS) vs. peripheral nervous system (PNS)), device functionality (recording, stimulation, or drug delivery) and invasiveness of electrode design. Interfacing with neurons either to record their action potentials or to deliver electrical current can be implemented in a variety of non-invasive and invasive methods. Electroencephalogram (EEG) and electromyography (EMG) are used

to non-invasively record electrical and magnetic brain activity, respectively[24,25]. While non-invasive approaches are generally safer, they suffer from low signal-to-noise ratio (SNR) and spatial resolution. Meso-scale recording using electrocorticography (ECoG) offers a superior solution for BCI applications with relatively greater amplitude, spatial resolution and frequency range since the ECoG grid is placed closer to the brain either on the surface or underneath the dura mater[26]. In contrast, penetrating cortical microelectrodes, such as microwires, planar Michigan electrodes and Utah electrode arrays, are inserted into the brain tissue to allow for the highest spatial resolution to record neural firing from a single neuron or a small group of neurons with high SNR, but not without insertion trauma and inflammation. Similarly, electrodes for recording or stimulating electrical activity from peripheral nerves can be non-penetrating such as a nerve cuff wrapping around a nerve fiber. Alternatively, they can be penetrating, like interfascicular arrays or regenerative mesh electrodes that reach an individual fascicle or axon for enhanced selectivity. Comprehensive reviews on the different types of recording and stimulation electrodes[27] have been reported by Patil et al.[28] and Kim et al.[29] .

1.C. Immune Response to Neural Implants

Invasive neural implants evoke a biological response upon implantation into the tissue. The penetration of intracortical electrodes can lead to mechanical tearing of the cells, vascular damage, blood-brain-barrier (BBB) rupture along with increased tissue strain due to tissue volume displacement[30–33]. Upon BBB disruption, plasma proteins such as albumin, globulins, and fibrin/fibrinogen are released, which trigger a cascade of inflammatory responses[34,35]. Hemoglobin released from breakdown of red blood cells causes an increased production of reactive oxygen and nitrogen species (RONS) which can have a detrimental effect by oxidizing cell lipids and proteins[36,37]. As a result of this increased oxidative stress, proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta(IL-1β) are upregulated which can promote neuronal degeneration and demyelination[37–39].

Additionally, within ~30 minutes of the implantation microglia cells, the resident immune cells in the brain, sense the injury induced chemotactic factors, and extend their processes to the implant surface[40]. Due to the relatively hydrophobic nature of common implant materials like metal, polymers, silicon and silica, plasma proteins, pro-inflammatory molecules and cytokines after BBB disruption are likely to nonspecifically adsorb onto implant surface and trigger activation of microglia. With additional help from monocytes that migrate towards the foreign body, activated microglia and macrophage begin to encapsulate the device[41], followed by astrocyte activation over the course of 2-3 weeks[42–45]. Presence of glia rich cellular sheath acts as a barrier and limits ionic exchange and excludes neurons from the electrode surface. In addition, the blood-derived macrophages and activated microglia and astrocytes continue to release pro-inflammatory factors that induce neuronal degeneration[46]. Reduction in the number of viable electrode site-interfacing neurons, persistent BBB leakiness and astrogliosis can contribute to deteriorating recording performance for chronic implantations, characterized by decreased signal-to-noise ratio (SNR) and number of viable units, along with increased impedance[47]. Moreover, sustained

brain micromotion against a static device that is several orders of magnitude stiffer than the neural tissue can further aggravate the inflammatory response over longer periods of time[48].

Another prominent aspect of the immune response is the proliferation of fibrous meningeal tissue to encapsulate regions of penetrating electrode arrays at the surface of the brain. Accounting as a major biological failure mode for over half of chronic array implantations, several factors can further affect the type of encapsulation formed along with the eventual extrusion of the implant[49,50]. Differences in vascular damage upon implantation can affect the rate and the degree of the infiltrating fibroblasts, dura regrowth, along with fibroblasts influx from arachnoid and dura mater[31,51–53]. Meningeal encapsulation is also a significant problem for sub- and epidural electrocorticographic electrode grids[51].

Electrodes implanted in the PNS also experience a foreign body response. A day after implantation, the acute response includes swelling of the axons and breakdown of the myelin, which has been linked to Wallerian degeneration[54]. During the first week after surgery, macrophages migrate to the implant site to scavenge and remove myelin and axonal debris. Simultaneously, a layer is formed around the implant composed mainly of monocytes and fibroblasts, which over time is further encapsulated within a fibrotic network. The thickness of the encapsulating layer corresponds to the increase in electrode impedance and stimulation threshold for peripheral nerve stimulation[55].

2. Biomolecular-based Approaches to Improve Biocompatibility

In addition to optimizing the intrinsic material device properties, introducing biological molecules that minimize the undesired immune response and promote neural tissue integration is a promising strategy. There are several players participating in the inflammatory foreign body response including but not limited to microglia/macrophages, astrocytes, pericytes, fibroblasts, blood cells and plasma infiltrates, all of which at the time of a CNS injury can have a detrimental effect on neuronal health around the implant site. Biological interventions can be designed to target these key players with the goal of reducing the inflammatory response and/or promoting neuronal growth to ultimately enhance the device-tissue integration. Different classes of biological molecules are engineered to implement these approaches and some general themes can be observed (Figure 1). These interventions are typically implemented in three main ways: 1) surface immobilization of biomolecules or biomimetics; 2) delivery of therapeutic agents/growth factors or 3) tissue engineering.

2.A Biomimetic coatings

Inspired by their growth-promoting properties *in vivo*, naturally occurring extracellular matrix (ECM) proteins, like laminin or collagen, have been utilized for supporting neuronal adhesion and growth but their effect is not neuron specific[56–61]. Laminin peptides that have specific neurite promoting function have been immobilized on neural electrode surface via electropolymerization of conducting polymers to improve neuron-electrode connection[62–64]. Transmembrane surface proteins like neuronal cell adhesion molecule L1 has demonstrated a promising increase in neuronal population when applied as a coating to camouflage the implant surface. Specifically, L1 coated silicon microelectrodes not only promoted

neuronal attachment but also inhibited microglial attachment and astrogliosis *in vivo* and were found to have more stable recording performance demonstrated by increased channel yield and SNR[65–68]. Influencing the local concentration of the reactive oxygen and nitrogen species (RONS) to reduce the oxidative stress around implant site is another strategy. Functionalization of a synthetic mimic of superoxide dismutase (SOD) on neural electrodes demonstrated a decrease in the local RONS level among other parameters *in vitro* and *in vivo*[69,70]. These results indicated a reduction in oxidative stress which led to a positive impact on neurons. To inhibit the non-specific adsorption of pro-inflammatory molecules released upon BBB rupture, anti-biofouling coatings comprised of hydrophilic zwitterionic polymers have been successful in reducing protein adsorption on electrode surface and inhibiting microglia response in comparison to uncoated surface[71,72].

2.B. Drug Delivery

Delivering anti-inflammatory, antioxidant and neuroprotective pharmacological drugs in conjunction with electrode implantation is another strategy to reduce local inflammation. Systemic delivery of agents such as dexamethasone[73,74], resveratrol[75], or melatonin[76] to name a few, have shown a decrease in the inflammatory tissue response and improvement in neuronal health around the implant. In a chronic *in vivo* study, daily melatonin injections for 16 weeks had a direct functional outcome of improved recording performance as compared to control[76]. While these effects are promising, the biggest limitation in systemic delivery is the potential unwanted side effects. Drug eluting coatings in the form of synthetic or biological films[77,78], hydrogels[79], polymers[80], nanoparticle carriers[81] can thus provide a localized therapeutic effect by releasing the drug via diffusion, coating degradation or swelling of the hydrogel containing the drug[82]. For precise temporal and spatial control of the drug release, pharmacological agents like dexamethasone and melatonin can be embedded in conductive polymers (CPs) like poly(3,4-ethylenedioxythiophene) (PEDOT) and polypyrrole coatings or hydrogels which can release the drug on demand after an electrical stimulus is applied[83–86]. From a materials perspective, carbohydrates can be potential candidates for drug delivery carriers given their general biocompatibility, stability and charged side-groups which can be utilized as counter ions for CP-based coatings for electrical release.

2.C. Tissue Engineering

The advancements in neural tissue engineering focused on promoting repair and regeneration through nervous tissue connectivity after injury is highly relevant for improving the device-tissue integration. Tissue engineering strategies can help to dampen the inflammatory response via immunomodulation, improve the acute and chronic tissue tolerance to an implanted foreign device or support innervation at the neural interface through tissue engineered electrode coatings[87].

In the PNS, traditional neural electrodes used for recording neural activity employ different designs to conform with the nerve geometry, fascicular arrangement and the fiber composition, with different trade-offs between invasiveness and signal-to-noise ratio along with spatial resolution[88] . Strategies involving tissue engineering incorporate an infrastructural material component which can provide the ideal environment for structural

regeneration along with the required signaling cues to support cellular growth. Incorporating features which mimic the architecture of native nerve tissue can further provide physical guidance cues for axonal extension after injury. Some examples include multi-channeled conduits[89] which mimic the nerve fascicles or longitudinally aligned porous materials that can support the proliferation of cells[90]. A comprehensive review on the design, materials and fabrication of nerve guide conduits can be found elsewhere[91].

Hydrogel-based scaffolds can similarly be fabricated with synthetic polymers[92,93] or biologically derived materials such as decellularized ECM[94] to match the mechanical modulus of the tissue. In addition, these scaffolds can serve as a reservoir for various agents such neurotrophic factors which regulate axonal growth and survival or stem cells that can replace the lost or injured cells along with inducing neuroprotection[95]. The scaffolds can also be fabricated using ECM based components, namely either proteoglycans (such as glycosaminoglycans) or fibrous proteins (e.g. collagen, fibronectin, laminin)[88,96]. These materials can be hosts for gene delivery for inducing gene expression in a subset of cells which can then produce therapeutic proteins of interest[97,98]. Additionally, these approaches can be combined with multi-electrode systems to enable recording and stimulation while promoting regeneration of the tissue surrounding the implanted device [18,99,100].

2.D. Limitations of Current Biomolecular Strategies

Several protein-based biomimetic coatings, such as laminin and L1 have proven themselves successful in promoting neurite outgrowth or reducing inflammation *in vivo*. However, due to the fragile nature of proteins, their production, purification, handling, and storage can be challenging. Protein molecules may degrade during this process, rendering their bioactivity non-functional. Additionally, immobilization of proteins can make the surface susceptible to further non-specific protein adsorption from BBB leakage upon *in vivo* implantation. Protein biofouling on implant surface is considered undesirable for cortical neural electrodes since its implicated in amplified immune response and glial scarring.

Intracortical drug-delivery based approaches demonstrate reduction in local inflammation and degeneration around the implant site, as compared to the systemic delivery alternatives. However, the therapeutic effect is typically limited to acute applications and is restricted by the reservoir capacity of the drug-eluting system. Precise spatial and temporal release of the drug is another major challenge and failure to control the release may result in leaks and undesired side-effects[101]. The presence of rigid and bulky components in the drug delivery system and the potential material degradation may evoke an additional inflammatory response.

Hydrogel-based engineered scaffolds are capable of drug elution and promoting tissue integration, however, in their hydrated form they can dramatically increase the device footprint and even displace the native tissue[88]. There are several factors to consider when selecting the polymer for fabricating the hollow nerve guidance conduits and commonly used polymers have their limitations. Biologically derived material such as collagen, has shown promising performance akin to autografts for short nerve gaps, but can potentially evoke an immune response[87]. Biodegradable synthetic polymers like poly (L-lactic acid) (PLLA), polyglycolic acid (PGA), and poly(lactic-co-glycolic) acid

(PLGA) allow more control over design while avoiding donor site morbidity and immune rejection problems. However, they can experience neuronal tissue mismatch, release acidic by-products upon degradation and lack native topography and cellular adhesion sites[87,102]. Introducing biological neurotrophic factors, ECM components, or stem cells may enhance the biocompatibility of engineered conduits integrated with neural devices but their effect on device functionality and chronic stability is still being investigated[103].

2.E. Untapped Potential of Carbohydrates

Carbohydrates, varying from linear polysaccharide chains to complex branched structures, attached to protein and lipid anchors in the body are involved in numerous functions in the body. Their immense structural heterogeneity supports the wide range of functionality, making them a valuable tool for engineering novel neural implant technologies for a broad range of applications. The general high abundance of hydroxyl groups in polysaccharides imparts a hydrophilic property to these structures which can be harnessed in applications that benefit from hydrophilic implant surfaces. Some polysaccharide chains contain charged groups which can be employed to bind other charged molecules through electrostatic interactions. The general hydrophilicity of most carbohydrates allows for easy processing and handling in aqueous mediums along with increased stability, relative to proteins. Moreover, carbohydrates are involved in cell-cell signaling, host-pathogen interaction along with other inflammatory pathways. Incorporating glycans in neural implant systems can thus facilitate modulation with the local environment through specific ligand-receptor binding to amplify or dampen certain desired pathways.

3. Role of Carbohydrates in the CNS

3.A. Introduction to Glycoconjugates

Glycosylation, the conjugation of glycans to lipids (glycolipids) and proteins (glycoproteins), is a complex process which results in a library of complex, heterogenous glycoconjugate structures. The glycan chains are composed of monosaccharide units which may be comprised of fucose, galactose, glucose, N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), mannose, glucuronic acid, and xylose. The structural heterogeneity can be expressed through different combinations of the monosaccharides, glycosidic linkages between monosaccharide units and/or the different linkages between the saccharide unit and the protein or lipid core. The addition or removal of monosaccharide units from the core polysaccharide altering the chain length can also contribute to this heterogeneity. The resulting large collection of diverse structures allows for a wide range of function at different stages of development for different cell types. On the surface of various cell types, the glycolipids and glycoproteins form a layer called glycocalyx which provides structural stability, a reservoir for sequestered growth factors, and regulates cell recognition, communication and adhesion[104,105] among other functions. These glycoconjugates can be divided into four main classes: 1) mucins comprised of glycoproteins with bulky O-linked glycan side chains 2) glycoproteins with N- and O-linked glycans 3) glycolipids with ganglioside attached to ceramides 4) proteoglycans with negatively charged glycosaminoglycan (GAG) side chains[105]. The bulk of the glycocalyx is comprised of glycoproteins and proteoglycans which will be focused on in this review.

3.B. Glycoproteins

Glycoproteins can either be situated in the transmembrane or anchored to the cell surface via glycosylphosphatidylinositol (GPA), or in the extracellular matrix (ECM) and serve as recognition molecules on various cell types. Glycoproteins are critical for neurite and astrocytic outgrowth, neurogenesis, myelin formation and maintenance, cell signaling, and synaptic plasticity[6]. This wide range of functions is facilitated by two broad classes of glycoproteins: N-linked and O-linked glycans. The major difference arises from the linkage of a core polysaccharide structure to either an asparagine residue to form N-glycans or serine/threonine to form O-glycans, respectively. Normal N-glycosylation is critical for healthy brain development and disruptions to the glycosylation process can lead to a broad range of neurological diseases identified as congenital disorders of glycosylation[106].

3.B.1 Cell adhesion molecules—The immunoglobulin (Ig) superfamily of cell adhesion molecules (CAMs) are abundantly present cell-surface glycoproteins involved in axonal growth and guidance in the developing brain[107]. While there are numerous Ig CAMs members, this section will briefly review the closely related neural cell adhesion molecule (NCAM) and L1.

NCAM was the first adhesion molecule to be characterized in the retina and the brain where it was found to mediate cell adhesion functioning via homophilic and heterophilic interactions with CAM molecules and other IgG family of adhesion molecules, respectively[108–110]. Comprised of five IgG-like regions followed by two fibronectin type III domains (shown by figure 2), NCAM can interact with other components of the extracellular matrix (ECM) such as proteoglycans and their GAG chains, and various other growth factor receptors[111–113]. Through these interactions and other molecular pathways, NCAM mediates cell adhesion, neurite outgrowth or migration, and synaptic plasticity[114]. Polysialic acid (PSA) is the predominant carbohydrate attached to the protein core of NCAM via N-glycosylation. A linear homopolymer comprised of α2,8-linked sialic acid, PSA can extend to 50-200 units, providing an additional layer of information to its protein scaffold NCAM which is involved in cell-cell adhesion and interaction[115–117]. Specifically, the carboxyl groups with negative charge can increase the hydration volume around the molecule, thus enhancing the steric hindrance which can inhibit hemophilic interactions between neighboring cells and provide an anti-adhesive property[117–120]. PSA expression is development-dependent and is mainly upregulated during early development and after a lesion when axonal and dendritic regeneration occurs.

L1 cell adhesion molecule is a 200-220 kDa transmembrane glycoprotein comprised of six Ig-like regions followed by five fibronectin type III regions. Through these regions, L1 can interact with several binding partners in cis (within same plasma membrane) or trans (adjacent cells) and be involved in development[121]. Specifically, L1 can promote neurite outgrowth by homophilic adhesion via its carbohydrate sidechains on the IgG subunits[122,123]. Purified L1 serves as an excellent substrate for promoting axonal growth and has been utilized as a biomimetic coating extensively by Cui group[49,65,68,124–127]. Through immobilization of L1 protein onto the microelectrode surface, the growth promoting property was harnessed to promote a significant increase in the neuronal density around

the implant site as compared to uncoated electrodes and reduce inflammatory responses. The functional outcomes were improved recording performance in chronic applications[65].

3.B.2 Oligomannosides—Oligomannosides are another class of N-linked glycoproteins comprising of Man9, Man8, Man6 and Man5 which are formed by sequential removal of mannose units from the core structure as shown in the Figure 2 (C)[6]. In other tissues, oligomannosides are eliminated during processing to produce mature N-linked glycans, however, in the brain they are carried to the cell surface on recognition molecules, such as NCAM and L1. Introduction of additional oligomannosides on the electrode surface may further promote neurite attachment and outgrowth[6,128].

3.B.3 Myelin associated glycoprotein (MAG)—MAG is a transmembrane glycoprotein present in the periaxonal Schwann cell and oligodendrocyte membranes of myelin sheath. The structure is illustrated in Figure 2 (A). Its localization in myelin implies its role in facilitating interactions between glia and axons along with aiding the formation and maintenance of myelinated axons[129]. MAG is known to collapse axonal growth cones and inhibit neurite outgrowth in a sialic acid binding-dependent manner[130,131]. Interestingly, it was shown to inhibit growth in the adult, whereas in the early developmental stage it enhanced *in vitro* neurite outgrowth[132], indicative of the environmental change making regeneration less conducive[133]. Given that their expression is enhanced during early developmental growth phase, introducing them at the neural interface may signal the nearby tissue to initiate growth and development, despite the presence of a foreign body. Alternatively, insertion of enzymatic factors that inhibit MAG expression around implant site in adult rodents for injury models may be useful in promoting axonal regeneration.

3.B.4 Human natural killer glycan (HNK1)—The HNK1 carbohydrate epitope is comprised of sulfated glucuronic acid linked to a galactose and located on both N-linked and O-linked glycoproteins as well as glycolipids via ceramide linkage as shown in the Figure 2 (D)[6]. HNK1 is present on several N-glycan recognition molecules, including Ig superfamily glycoprotein P0 in peripheral nerves which plays a role in the formation and maintenance of myelin[134]. Upon binding to its receptors, HNK1 is thought to be involved in development. ECM glycoproteins laminin, which is expressed during different developmental stages, and chondroitin sulfate proteoglycans (CSPG), bind to HNK1 to mediate neuronal cell adhesion and neurite outgrowth[135,136]. Like growth-promoting functions of other glycoproteins, introduction of HNK-1 on the neural electrode surface can potentially promote the binding to its nearby receptors and facilitate neurite growth.

3.C. Glycolipids

Sialylated glycosphingolipids, specifically gangliosides, are the most abundant family of glycoconjugates in the brain, consisting of glycan attached to a ceramide lipid. As shown in Figure 2 (E), the glycan is linked via glycosidic linkage to the ceramide comprised of long-chain base and fatty acid amide. There are four main complex ganglioside structures identified which constitute a majority of the structural diversity: GM1, GD1a, GD1b and GT1b[137]. All four gangliosides share the same neutral glycan core (Gal β1–3 GalNAc β1–4 Gal β1–4 Glc β1–1 Cer) while the difference lies in the varying numbers of

sialic acids attached to the internal and terminal galactose residues. Approximately 80% of the total glycan mass in the brain is attributed to glycosphingolipids highlighting the importance of their functionality in the brain. Multiple studies on genetic mice models clarified that complex brain gangliosides are not vital for neuronal development (proliferation, differentiation, migration or synapse formation), but are required for optimal myelin formation, axon-myelin interactions, long-term axon stability and neuronal excitability[138–141]. Mice with sialytransferase mutants exhibited motor deficits along with cognitive disabilities which closely matches observations in human congenital disorders[142].

Specifically, complex gangliosides GD1a and GT1b promote healthy and stable axon-myelin interactions by acting as a receptor for MAG which is selectively expressed by myelinating cells in the CNS and PNS[143,144]. In addition, complex gangliosides were reported to enhance not only the ability of MAG to protect axons from short-term toxic insults but also enhance survival of nerve cells in the presence of these insults[145,146]. However, since MAG is also involved in inhibiting axon regeneration, binding to its ganglioside receptors may transduce signals which can inhibit axonal outgrowth after injury. To this end, treatment with sialidase enzymes which cleave the ganglioside chains at the site of spinal cord injuries were shown to promote improved axon regeneration, making inhibitory gangliosides a pharmacological target[147,148]. Another route to promote growth has been through exogeneous addition of GM1 or GM3. For instance, GM1 was reported to enhance the activation of high-affinity nerve growth factor receptor (TrkA) along with facilitating the association of laminin, β1-integrin, TrkA and intracellular Lyn to stimulate neurite outgrowth[149–151]. Other studies have also shown GM1 to achieve outgrowth by modifying calcium influx or by non-specifically altering the structure of the membrane milieu[152–154]. In human trials where GM1 was injected to promote neural outgrowth and regeneration, alleviation of motor deficits in Parkinson's disease was observed but the therapeutic effect in patients with stroke and spinal cord injury was limited[155–157].

3.D. Glycosaminoglycans (GAGs)

Glycosaminoglycans are linear, repeating chains of sulfated disaccharide units with a negative charge. They are widespread through the body and their biological function is determined by the molecular composition, linkage between units, degree of sulfation and their attachment to the protein core[158]. There are five main types of GAG chains with distinct combinations of disaccharide units, composed of an amino sugar and galactose or uronic acid with differences in sulfation as illustrated in Figure 3. These include 1) chondroitin sulfate 2) dermatan sulfate 3) keratan sulfate 4) heparan sulfate 5) hyaluronan. These GAG units are then covalently attached to a core protein through a tetrasaccharide unit composed of one unit of GlcA, two units of Gal and a unit of xylose linked via serine/threonine (O-linkage) or via asparagine (N-linkage)[158,159]. Together, the repeating disaccharide chains, polysaccharide core and the protein scaffold compose the proteoglycans. The strong negative charge in GAGs is primarily imparted by the sulfate and uronic acid groups.

Hyaluronan is an exception since it is not bound to a protein core and simply exists as a large polymer comprised of GlcA and GlcNAc. Heparan sulfate is the most prominent

GAG chain of proteoglycans, constituting over 50% of the attached chains, whereas chondroitin sulfate, dermatan sulfate and keratin sulfate are the remainder side chains[159,160]. Specifically, in the CNS, chondroitin sulfate and heparan sulfates are the major GAGs coupled to different protein backbones. The heterogeneity expressed through the different disaccharide combinations, linkages, extent and location of sulfation, protein core are useful in delegating different functions to proteoglycans in the CNS[160].

As part of the glycocalyx canopy on the cell surface, the long-repeating polysaccharide units with negative charge influences interactions with surface receptors and ligands[161]. Further, this charged, mesh-like glycocalyx acts like a barrier for maintaining vascular homeostasis, prevents adhesion of circulating inflammatory molecules and platelet aggregation and can reduce oxidative stress[162,163]. A recent study found the endothelial glycocalyx to be denser in the cerebral capillaries as compared to cardiac and pulmonary capillaries alluding to the vasculo-protective properties relevant for the blood-brain-barrier (BBB)[164]. These various roles of glycocalyx in different physiological contexts are a direct function of the structural diversity of the glycoconjugates.

3.D.1. Chondroitin Sulfates (CS)—CS disaccharides are comprised of N-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA). Chondroitin sulfate proteoglycans (CSPGs) are components of the perineuronal net (PNN) which is a collection of extracellular matrix components encapsulating the cell surface. The CSPGs in the lectican family comprising of aggrecan, versican, neurocan and brevican, are important in the CNS[165]. While all four members of the lectican family are expressed on neurons, neurocan and versican are additionally present on astrocytes and cells of oligodendrocyte lineage, respectively[166–168]. In addition to the lectican family, neuron-glial antigen 2 (NG2) is another important CSPGs which is expressed on the cell surface of activated microglia, macrophages and oligodendrocyte precursor cells[169]. NG2 was implicated in inhibition of neurite outgrowth and restriction of axonal regeneration via the protein core[169,170]. Other proteoglycans which may contain a combination of CS and/or keratan sulfate, and/or dermatan sulfate sidechains are phosphocan and decorin, respectively. Phosphocan is thought to be involved in brain regions with active proliferation with expression limited to those regions as compared to other widespread CSPGs like neurocan[171–173]. Decorin is expressed by both neurons and astrocytes and reported to have anti-inflammatory and anti-fibrotic properties[174,175].

Differential sulfation pattern can lead to distinct CS disaccharides subtypes. The substitution of GalNAc residues with sulfate groups occurs either at the carbon-4 (C-4) and/or at the carbon-6 (C-6) position, or sulfate groups are substituted at the C-2 position in the uronic acid, leading to CS-A, CS-C, dermatan sulfate (previously known as CS-B), CS-D and CS-E [176,177]. While the protein core is similar, this additional layer of diversity in sulfation pattern allows for diverse binding properties.

The structural heterogeneity allows for complex and at times contradictory involvement in cell proliferation and differentiation, neural migration, axon guidance and synapse formation. In early development phases, CSPGs serve as inhibitory barriers which repel growth cones along with inhibiting a variety of growth-promoting molecules like fibronectin

and L1. After CNS injury, CSPG expression (neurocan, phosphocan and NG2) was reported to be upregulated and involved in glial scarring[166,170,178,179]. Treatment with chondroitinase ABS (ChABC) after injury was shown to attenuate the CSPG inhibitory activity suggesting their importance as a pharmaceutical target in treating CNS injury [176,178,180,181].

However, CSPGs were also shown to play a role in binding to growth factors along with promoting neurite outgrowth *in vitro* when presented to neurons as a uniform substrate as opposed to electrostatic molecular barriers[182,183]. In addition, the immobilization of highly sulfated CS subtypes (CS-D and CS-E) showed higher neurite outgrowth *in vitro*, alluding to the potential role of sparsely sulfated CS in inhibiting growth[184–186]. The precise regulation of the side chain structural diversity through sulfation pattern, allows for these contrasting interactions, which occur in a temporal and spatial manner[176]. The presence of CSPGs on PNNs was shown to be implicated in regulating neural and synaptic plasticity[187]. In addition, insoluble and highly charged complexes of CSPGs are thought to be responsible for ion homeostasis, synaptic stabilization and neuroprotection[188,189]. A comprehensive review on the contradictory roles of CSPGs in nervous system development can be found in Mencio et al[190].

3.D.2. Keratan Sulfates (KS)—KS is composed of galactose (Gal) and N-acetylglycosamine (GlcNAc) with three different types of linkages between the oligosaccharide and core protein in the proteoglycans. These different linkages correspond to different KS subtypes: 1) KS I identified primarily in the cornea; 2) KS II identified in cartilage 3) KS III identified in the brain tissue[159]. There is a varying extent of elongation and sulfation patter in these different KS subtypes allowing for different physiological functions. Similar to the inhibitory function of CSPGs, KS was also reported to restrict neural plasticity after CNS injury [191]. Digestion of KS and CS chains were also shown to have comparable effects on the functional recovery *in vivo* suggesting that they both may work in the same inhibitory pathways for axonal regeneration[192].

3.D.3. Hyaluronan (HA)—HA is composed of long unbranched, non-sulfated repeating disaccharide units of GlcA and GlcNAc which can form up to 25,000 disaccharide units. The size, concentration and localization of HA determines its distinct physiological functions. HA has been implicated in altering tissue elasticity and hydration along with creating cell-free spaces considered crucial for cell migration. Through specific binding with transmembrane receptors, HA can initiate cell signaling[193]. Interestingly, the contribution of HA to repair after CNS injury is contradictory. The low molecular weight-HA (LMWHA) in the range of 10-500kDA can play a pro-inflammatory role by binding to cell surface receptor CD44 which triggers the upregulation of chemokines and cytokines. In addition, LMWHA were shown to inhibit axonal regeneration in the CNS[194,195]. In contrast, high molecular weight-HA (HMWHA) in the range of 800 kDa - 1.2MDa play an anti-inflammatory role[196]. The localization of HA with CSPGs in the perineuronal nets also plays a role in facilitating synaptic plasticity[197]. Therefore, HMWHA can be utilized for developing bioactive neural coatings to provide an anti-inflammatory effect on the tissue surrounding the implant site.

3.D.4. Dermatan Sulfates (DS)—DS are composed of either GlcA or iduronic acid (IdoA) and GalNAc and are stereoisomers of CS disaccharide, which was initially termed as CS-B. CSPGs like versican contain both CS/DS chains, expressed in the brain, can facilitate leukocyte trafficking and inflammatory response by binding to L- and P-selectin adhesion molecules[198].

3.D.5 Heparins and Heparan Sulfates (HS)—HS is comprised of GlcA or IdoA and their functional specificity is also a function of the sulfation pattern. It's through these sulfation motifs, HS chains can interact with a wide range of proteins, including heparin-binding growth factors, morphogens, chemokines, proteases and their inhibitors along with ECM proteins in a structure-dependent manner[199–201]. HSPGs are known to be associated with the plasma membrane either as a glycosyl-phosphatidylinositol (GPI)-anchored proteins such as glypicans or as a transmembrane protein such as syndecans. Syndecan 2, syndecan 3 and agrin are expressed in the dendritic spines and synapses, along the axons, and in the synaptic basal lamina respectively. Expression of these HSPGs at the synapse alludes to their function in regulating synaptic efficacy[202]. The glypicans are expressed at the synapse and are involved in tumor development and demyelinating diseases. HSPG from the ECM can also be secreted, such as perlecan which plays a role in maintaining the BBB[203,204] HSPGs are thus abundantly present in the mammalian brain and plays a developmentally regulated role in neurogenesis, axon guidance and synapse development[205,206]. Incorporating HS in hydrogel scaffolds for stem cell differentiation can be useful since HS can preserve the biological lifetime of the growth factors through electrostatic binding. Another application could focus on the surface immobilization of heparan sulfate for blood-contacting surface to reduce the likelihood of thrombosis.

4. Polysaccharide-based Biomaterials for Neural Engineering Applications

4.A Neural Electrode Surface Modification

The interface between the nervous tissue and the neural implant is a dynamic environment which facilitates bidirectional interaction for neural recording, stimulation and biosensing. There is a complex interplay of biotic and abiotic factors which can contribute to a host of failure mechanisms for chronic implantations. For neural tissue interface applications, biomaterials that enhance the implant function by either actively improving the device capability or by dampening the local inflammation are ideal candidates. In an effort to increase efficacy of recording and stimulating neural electrodes, a vast body of research has been dedicated towards doping conducting polymers (CP), like poly(pyrrole) (PPy), poly(aniline) (PANI), polythiophene (PTh) and its derivatives including poly(3,4-ethylene dioxythiophene) (PEDOT). Along with being biocompatible, these CPs provide decreased electrode impedance and increased charge injection capability[62,63,207]. While they offer excellent electrical properties and softer mechanical modulus, CPs are still foreign materials that cannot escape the inflammatory tissue responses. Biological dopants can impart additional biocompatibility and biological functionality to the polymer composite and can be varied to obtain different surface roughness, morphology along with chemical and biological functionality. Introducing polysaccharides, specifically, can be advantageous given their critical role in facilitating interactions between cell types. Moreover, their side groups

often contain charged moieties which can be utilized for facile electrostatic binding with oppositely charged conducting polymers. Thanks to the abundance of hydroxyl groups, they can also impart hydrophilicity to the surface which can resist non-specific protein fouling, the first step that initiates the foreign body response. This section summarizes a few such applications for key polysaccharides of interest (Table 1).

4.A.1 Chondroitin Sulfate—Inspired by its structural integrity and abundant negative charges, CS was utilized as a dopant for PPy and PEDOT on neural electrode arrays. Harris et al. found the electroactive surface area of the PEDOT-CS modified electrodes to be comparable to PEDOT para-toluene sulfonate (PEDOT-pTS), which is known to have high charge injection capability and promising neural recording performance[208,209]. This study provides useful insight into the effect of PEDOT-CS coating for impedance but further investigation into *in vivo* recording quality is necessary. In a different study, conductive PEDOT dispersion doped with PSS, CS, HA, HS was assessed for cell viability and neuroregenerative processes *in vitro*. CS emerged as the ideal bioactive dopant with higher conductivity and increased cellular attachment and proliferation of human neuroblastoma cell line (SH-SY5Y) and human astrocytoma cell line CCF-STTG1, as compared to the HA and HS. In addition, PEDOT:CS demonstrated a neuroprotective effect where SH-SY5Y cells were partially protected from hydrogen peroxide induced cell death. Thus, PEDOT:CS serves as an attractive choice with optimal bioactivity for neural recording and stimulating implants[210].

4.A.2 Hyaluronic acid—As a naturally occurring ECM polyanionic polysaccharide in the brain with anti-inflammatory properties towards astroglia cells, hyaluronic acid (HA) is an appealing candidate for biomimetic electrode coatings to increase biocompatibility with the neural tissue. HA has been especially useful in enhancing the biocompatibility of conducting polymers for *in vivo* applications since these conducting polymers while offering excellent electrical properties, can undergo redox reactions in the physiological environment. Lee et al. utilized HA to synthesize pyrrole-hyaluronic acid conjugates (PyHA) and electrically polymerized the conjugate on silicon microelectrodes and iridium microwire electrodes. After 3-week *in vivo* implantation in the motor cortex, coated iridium wires had decreased glial activation as compared to the uncoated microwires[211]. The hydrophilicity induced anti-fouling property of HA was utilized in a study where polydopamine (PDA) was electrochemically deposited in the presence of HA onto the electrode surface, with no substantial increase in impedance. Significant resistance to protein adsorption and attachment of fibroblasts was observed in PDA/HA coated samples as compared to controls. Scar tissue thickness around modified electrodes after subcutaneous implantation was also significantly reduced suggesting the biocompatibility of this coating for implantable electrodes[4] as shown in Figure 4 (A).

HA can also be electrospun into nanofibers that comprise of ultra-low concentration of carboxylated multi-walled carbon nanotubes (MWCNT). The MWCNT are incorporated to reduce impedance and increase the charge capacity of the HA fibers for safe and effective delivery of current for stimulating neurons. Dorsal root ganglion cell growing on these substrates were reported to have longer neurite lengths after receiving 200 mV/mm

electrical stimulation for at least 30 minutes, as compared to the unstimulated controls[212]. In another study, chemical oxidative polymerization was used to synthesize HA-doped PEDOT nanoparticles, which were then incorporated into a PEDOT-HA/poly(L-lactic acid) (PPLA) composite film[213]. These films showed favorable electrochemical stability and were used to deliver electrical stimulation to PC12 cells which showed increased neurite growth under stimulation conditions, as compared to control. These studies emphasize the utility of polysaccharides in enhancing the biocompatibility of the neural device coatings without compromising the electrical conductivity to promote seamless neural tissue-device integration along with nerve tissue regeneration.

4.A.3. Chitosan, Alginate, Agarose—

Chitosan is the deacetylated derivative of the second most abundant and naturally occurring polysaccharide chitin, a major component of crustaceans, insect exoskeletons, bacterial and fungal cell walls. It is composed of β-(1,4)-linked *N*-acetyl-glucosamine units with positively charged amino (-NH_2) groups. Chitosan has been utilized heavily in several biomaterial applications thanks to its biocompatible, biodegradable, nontoxic, mucoadhesive, antioxidant, antimicrobial, anti-thrombogenic properties, to name a few[214]. A variety of chitosan derivatives can be synthesized to create chitosan-based nanoparticles[215,216], fibers[217,218], films[219] and gels[220,221] for neural tissue engineering. Chitosan and its derivatives were reported to have a neuroprotective effect for Alzheimer's and Parkinson's disease, stroke, injury, sclerosis among others. Comprehensive review on the topic have been discussed elsewhere[222,223].

Like other polysaccharides discussed in this section, chitosan can also be used as a dopant for electroactive coatings with bioactivity. In one study, electroactive hydrogel was fabricated by grafting oligoaniline to chitosan and epoxidized by reacting with epichlorohydrin which resulted in conductivity around 10^{-2} S/cm. By adding oligoaniline, the compression strength of the hydrogel was enhanced and the degradation rate was reduced[224]. Such coatings can be applied onto neural electrodes to reduce the stiffness of the electrode surface in contact with the tissue and potentially enhancing the integration. Chitosan can also be electrodeposited on a carbon fiber and encapsulate glucose oxidase for glucose sensing using background-subtracted fast-scan cyclic voltammetry in live brain tissue[225]. Another unique application of chitosan-based coating is the visualization of the neural interface device placement enabled by the intrinsic fluorescence of chitosan. Rauhala et al. validated the use of chitosan-coated probes in rodent brain where they introduced the coated probes in a spatially restricted manner using the fluorescence as a visual marker[15] as illustrated in Figure 4 (D – F).

Sodium **alginate** is a naturally occurring hydrophilic polysaccharide resembling the extracellular matrix in the body. In a scaffold form, it can enhance cellular adhesion and proliferation and when combined with conductive polymers such as PPy and PEDOT, it can serve as conductive hydrogel layer in neural electrode devices. When placed between the hard silicon-based probe and the soft brain, it can act as a mechanical buffer and provided controlled release for drug delivery[226,227]. Conductive alginate hydrogels can be further functionalized with arginine-glycine-aspartic acid (RGD) for cochlear implant coatings to enhance device performance and the biological stability of the inner ear[228]. Similarly, polyacrylamide-alginate hydrogels can be embedded with silver flakes to manufacture a

composites with high electrical conductivity, low Young's modulus and high-stretchability for soft, wearable bioelectronic designs[229]. Alginate-based hydrogel was also integrated with polymer-based fibers to develop a hybrid multifunctional probe for long-term neural sensing and recording as illustrated in Figure 4 (B) and (C). The alginate not only imparted a brain-like softness to the probes but also mechanical robustness along with long-term chemical stability in physiological *in vivo* environments and ease of interfacial integration with polymer fibers[12].

Like alginate, **agarose** is another natural polysaccharide polymer derived from algae which resembles the ECM, making it an attractive candidate for hydrogel scaffolds. Agarose gels have served as *in vitro* brain phantom models for testing electrode performance and imaging studies due to its ability to emulate the poroelasticity of the brain[230]. An agarose-carbon nanotube hydrogel composite functionalized with anti-inflammatory molecules showed reduced inflammatory markers *in vivo*, demonstrating the composite material's potential as a bioactive coating for microelectrodes[231].

4.B Drug delivery

Delivery of therapeutic agents at the interface between implanted device and local neural tissue can help in managing the inflammatory response and reduce likelihood of device failure. The impermeability of the blood-brain-barrier poses a significant challenge for drug delivery in treatment of neurological diseases which has inspired delivery vehicles capable of surpassing this boundary. Some of these strategies include but are not limited to delivery of viral vectors, nanoparticles, exosomes along with use of non-invasive techniques that enhance drug uptake and utilizing alternate routes of delivery such as internasal route. The polysaccharide-based materials discussed in this section are attractive candidates for drug delivery vehicles given their high stability, non-immunogenicity, biocompatibility and biodegradability[232]. In addition, the presence of various hydrophilic side groups such as hydroxyls, carboxyl and amino groups provides an anchor for functionalization with synthetic materials as well as to form non-covalent bonds with biological tissues[233] (Table 2).

4.B.1 Chondroitin Sulfate—As a naturally occurring anionic polysaccharide that is abundant in cartilage, skin, nerve tissue, ECM and blood vessels, chondroitin sulfate (CS) has been utilized as drug delivery vehicles with various applications for cancer-targeted therapeutics, osteoarthritis, and joint-cartilage repair to name a few[234]. Work in this area includes but is not limited to the formulation of CS-based nanoparticles, micelles, hydrogels, complex films, and microcapsules which harness the biocompatibility and biodegradability of CS[235]. Such carrier systems can be conjugated with CS which can then facilitate the crossing of the blood-brain-barrier and delivery drugs to the CNS. Another benefit of employing CS-conjugated delivery systems is the ability to target specific receptors of interest, specifically, CS has been used for targeting CD44 receptor which are highly expressed in brain tumors[236]. One study locally delivered temozolomide loaded CS-nanoparticles which crossed the BBB and was taken up by the tumor cells via CD44 receptor targeting and endocytosis[237]. Another study injected CS-conjugated superparamagnetic iron oxide nanoparticles into the substantia nigra of rats and found

that CS-conjugated particles surrounded neuronal cell bodies, dendrites and synapses and demonstrated reduced endocytosis compared to control due to their interaction with the peri-neuronal nets[2]. They visualized the localization of these particles using transmission electron microscopy (TEM) shown in Figure (5A).

Chondroitin sulfate can also be used to improve the efficacy of the delivery system. In some applications, CS has been used as a reducing and stabilizing agent for metal nanoparticles by inhibiting their agglomeration via electrostatic repulsion and steric hindrance[238]. The side groups of CS can also be modified with hydrophobic chains which can then self-assemble into nanometric carriers with a hydrophobic core for encapsulating insoluble drugs[239]. Exogenous coating of CS on cationic DNA complexes (such as arginine peptides) used for gene delivery have demonstrated enhanced transfection efficiency for several cell types[240].

4.B.2 Hyaluronic Acid—Like chondroitin sulfate, hyaluronic acid (HA) is a major component of the ECM and has implications in diseased states and has been used for targeted therapeutic delivery for cancer applications. While HA-based tumor therapies are outside the scope of this review, the material-based approach taken by the studies reviewed in this section can be of interest for inspiring elution of therapeutic or genetic agents at the neural interface from the electrode surface.

CD44 is known to bind to both chondroitin sulfate and hyaluronic acid, carriers encapsulating anti-cancer drugs like temozolomide can be modified to bind to tumor cells expressing CD44 in the brain[241]. Similarly for treatment of glioblastomas, HA conjugated liposomes were formulated with doxorubicin and an *in vitro* study demonstrated that the HA moiety promoted preferential uptake and significantly enhanced chemotherapeutic potency in glioblastoma cells while eluding uptake in healthy cells[242]. Another study utilized the mucoadhesive property of HA to formulate a lipidic nano-emulsion containing two insoluble polyphenol drugs (resveratrol and curcumin) and demonstrated successful *in vivo* intranasal delivery for treatment of neurodegenerative disease. The mucoadhesive nature of HA helps to delay the mucociliary clearance of the drug carrier which was reported to enhance the absorption of the drug via the mucosal tissue[243].

In the realm of nanotechnology, the combination of carbon nanotubes (CNT) and HA has demonstrated an immense potential in drug delivery systems. Owing to their cylindrical shape, the CNTs can penetrate the BBB effectively while providing electrical conduction with attractive photothermal features[244,245]. Even beyond cancer applications, anti-inflammatory drugs can be encapsulated within CNTs functionalized with HA and anchored on neural probe surfaces for targeted local release. Delivered agents can help dampen the local immune response or modulate cellular activity to promote more seamless integration at the device-tissue interface.

4.B.3 Chitosan, Alginate, Agarose—Several studies have demonstrated the use of chitosan for drug delivery to the brain for gliomas, Alzheimer's disease, and Parkinson's disease, especially in the nanoparticle formulation. Owing to its chemical structure with a high number of cationic free amine groups, **chitosan** can be combined with anionic materials and provide good mucosal adhesion for intranasal or intravenous delivery routes as

nanoparticles[246,247]. In addition, chitosan is compatible with the layer-by-layer (LbL) self-assembly method which is used to fabricate functional surface coatings involving alternating deposition of multivalent compounds under aqueous conditions[248].

In a gene delivery application, 3,4-ethylenedioxythiophene (EDOT) was modified with amphiphilic chitosan and conjugated with reduced graphene oxide to self-assemble into a nanogel composite which hosted a nonviral gene vector consisting of neurotensin and polyethylenimine to target neurons upon release[249]. Chitosan was chosen here for its soft tissue-mimicking structure and ability to enable electrically controlled delivery based on sensitively switchable swelling/deswelling ability. The schematic in Figure 5 (B) illustrates the use of chitosan hydrogel for enabling the on-demand delivery of Mn^{2+} for high-resolution manganese enhanced magnetic resonance imaging[10] used in conjunction with deep brain stimulation. In addition, it can serve as a biocompatible component for electroactive hydrogels for controlled release of loaded therapeutic agents[250].

Alginate based hydrogels have also shown promise as a biomaterial since alginate can be used an immobilization matrix to embed living cells[251], serve as microcarriers[252] and as bulk material for complex scaffolds loaded with therapeutics[253]. Alginate hydrogels can also be embedded with poly(lactic-*co*-glycolic) (PLGA) nanoparticles loaded with dexamethasone. These matrices can be functionalized onto microfabricated neural probes which upon can locally delivery therapeutics for a reduced glial inflammation response[254]. One study demonstrated the use of ultra-high viscous (UHV) alginate with decreased immunogenicity and enhanced mechanical stability to encapsulate mesenchymal stem cells as a drug delivery system to protect auditory neurons (Figure 5 (C–G)). Used in conjunction with cochlear implants, alginate hydrogel encapsulation provided stability to the cells which produced neurotrophic factors *in vivo* and showed increased neuron survival and improved cochlear implant outcome[16].

Agarose has been extensively used to emulate the softness of the brain for testing the efficacy of drug delivery *in vitro*. One study utilized the principle of electrophoresis by applying a direct current electric field to deliver charged chemotherapeutic doxorubicin in a brain-tissue mimic agarose gel. The drug was loaded on a capillary-embedded electrode encased with flexible and transparent polydimethoxysilane (PDMS) and exhibited sustained release of the drug in the brain phantom[255]. Another group fabricated an electroactive aniline-pentamer colloidal cryogel based on agarose, alginate and chitosan which was loaded with dexamethasone and released on demand upon application of 0.5V[221]. This electroactive carbohydrate polymer cryogel can be applied as a coating for neural recording and stimulating electrodes with drug eluting properties.

4.C. Neural Tissue Engineering

Majority of the literature exploring the use of carbohydrate as biomaterials has been done in neural tissue engineering for tissue repair and regeneration applications. They are nevertheless worth reviewing here because knowledge learned from these studies can be applied to neural interface applications that either utilize neural tissue engineering principals to make implant more biocompatible or directly incorporate regenerating tissue onto the device (Table 3).

4.C.1 Chondroitin-sulfate—The heterogeneity of CS chains allows for a wide range of growth-altering functions which can be harnessed for tissue engineering applications. Due to their polyanionic nature, CS can electrostatically bind with polycations and growth factors via specific sulfation motifs. Specifically, CS chains bind to fibroblast growth factor 2 (FGF2) and brain-derived neurotrophic factor (BDNF) with high affinity, two growth factors of high interest. FGF2 is known to promote neuroprotection, neural stem cell (NSC) migration and proliferation, whereas BDNF mediates neuroplasticity, promotes neuroprotection and functional repair after brain injury[256–259]. Additionally, they both promote angiogenesis after injury[260,261]. Utilizing this property, Betancur et al. encapsulated NSCs with a CS-GAG matrix that selectively bound to and sequestered endogenous FGF2 when implanted in the rat frontoparietal cortex in a traumatic brain injury (TBI) model. The CS-GAG promoted the self-renewal and proliferation of NSCs and enhanced the neuroprotection of the brain tissue up to 4 weeks after TBI[262,263]. In a follow-up study, Latchoumane et al. investigated the chronic impact, up to 20 weeks, of CS-matrix laden with FGF2 and BDNF in a severe TBI rat model. They found that CS matrices promoted chronic neuroprotection, reduced presence of neuroinflammatory cells, enhanced local vascularization and recovery of gross motor function as compared to sham controls. In a related study, CS-A hydrogel encapsulating neural progenitor cells (NPC) was implanted in the stroke core in mice. CS-A was found to act via FGF2 to potentiate NPC-mediated angiogenesis and vascular remodeling, blood flow recovery as well as sensorimotor recovery after injury (Figure 6 (A–E))[9] For applications in the PNS, a 3D CS-methacrylate hydrogel (CSMA) seeded with NSCs implanted post spinal cord injury (SCI) demonstrated inhibition of reactive astrogliosis and facilitation of neural regeneration as compared to sham controls[264]. Collagen-GAG matrices even without the embedded NSC were shown to have neuroprotective effects via upregulation of anti-inflammatory IL-10 and GM-CSF, and downregulation of pro-inflammatory NF-kB, TNF-alpha, and IL-6 after surgical brain injury[265]. These results strongly support the regenerative and neuroprotective role that CS-based biomaterials, especially hydrogels, can play in neural tissue repair after injury.

One study elucidated the molecular mechanism through which CS-dependent neurite growth promotion is exerted when hippocampal neurons are cultured on CS coated substrates. They found that CS subtype D acts as a neurogenic, extracellular ligand for ECM integrin αVβ3 present on neuronal cell surfaces and associated with downstream pathway of integrin-mediated adhesion[184]. Another study showed that CS subtype E increases binding to brain derived neurotrophic factor (BDNF) and midkine which activates cell surface receptors protein tyrosine phosphatase and tyrosine kinase to stimulate neurite growth. The varied sulfation motifs are responsible for recognizing and binding to molecular elements on growth factors to modulate neuronal growth[266]. These findings provide novel insights into the CS-related machineries that modulate CNS development and regeneration, which can further guide the design of smarter biomaterials for neural tissue engineering or neural interface applications.

4.C.2 Hyaluronic acid—Hyaluronic acid (HA) has been widely used in several biomaterials applications for cartilage, nerve, and skin repair along with drug delivery

system due to its excellent biocompatibility, biodegradability, and highly tunable scaffold formation. It serves as an appealing candidate for peripheral nerve tissue engineering, supporting nerve outgrowth, differentiation, and proliferation. Hydrogels with HA can be mixed with other natural adhesive molecules like collagen I, laminin, or fibronectin to support neural cell growth[267]. Spearman et al. synthesized HA hydrogels with two different forms of methacrylate chemistries for soft-tissue engineering, showcasing the tunability of HA-based hydrogels to match the mechanical stiffness of the target nerve tissue. After modifying these hydrogels with collagen I and laminin, they noticed an improved neurite growth due to the presence of ECM components[88] In a similar study, Li et al. synthesized multi-component scaffolds with collagen, CS and HA which housed mouse NSCs, and subsequently *in vitro* neuronal differentiation was monitored. The scaffolds with collagen-HA and collagen-CS-HA showcased selective enhancement of neurogenesis, suggesting their suitability for brain tissue engineering therapy, as compared to the collagen only control[268].

Aside from hydrogels, highly porous nanofibrous scaffolds composed of electrospun HA and polycaprolactone (PCL) fibers can be engineered. These nano-scale fibers are suitable for neural tissue applications since they provide high surface area and porosity with small pore sizes which can mimic the extracellular matrix (ECM) and enhance cell migration and proliferation[269]. Entekhabi et al. demonstrated improved adhesion and proliferation of SH-SY5Y human neuroblastoma cells on PCL/HA scaffolds as compared to PCL scaffolds only, suggesting the improved biological property imparted by HA[270].

4.C.3. Heparan sulfate (HS)—Highly sulfated HS binds to several growth factors, signaling proteins and ECM components through its heterogenous sulfation motifs and regulates various developmental processes[271]. Given these functions, HS serves as an attractive candidate for tissue engineering applications which involve modulating cell fate via growth factor binding. To utilize this property, Zhang et al. engineered a collagen/heparan sulfate porous scaffold which was embedded with NSC to treat TBI in rats[272]. Two-months after implantation, they observed significantly improved regeneration of neurons, nerve fibers, synapses, and myelin sheath in the injured brain tissue along with reduced brain edema and cell apoptosis; these markers subsequently corresponded to markedly improved motor and cognitive functions. Incorporating heparan sulfate not only enhanced the regeneration inducing bioactivity of the scaffold, but also enhanced the mechanical properties of the collagen scaffold, which is crucial for housing the NSCs[272].

While the application of HS in formulating biomaterials is promising, it is riddled with challenges. The structural heterogeneity and the binding promiscuity of HS to proteins can have benefits in certain environments and adverse side-effects in others[273]. To precisely control the binding, one emerging approach is to synthesize HS molecules which mimic the natural sulfation patterns. These HS mimics can be produced in large amounts for systematic structure-function studies as well as for exploring their therapeutic potential. Chopra et al. prepared a polyethylene glycol-based hydrogel with synthetic HS-derived disaccharides to encapsulate human induced pluripotent cell-derived neural stem cells (HIP-NSCs)[274]. The synthesized HS disaccharide bound to FGF-2 and promoted a significant enhancement in proliferation and self-renewal, by preserving the FGF-2 which is known to have a short

biological life. Synthetic HS mimics can thus be engineered for controlling the retention and release of specific growth factors which regulate cell fate in regenerative environments.

Similarly, Malaeb et al. used alginate with varying degrees of sulfation as a HS mimic to investigate FGF-2 binding. Using these sulfated mimics, they constructed a layer-by-layer (LbL) on top of gold substrates via biotin-streptavidin interactions and characterized growth-factor binding. Mimics with higher degrees of sulfation showed increased FGF-2 binding and enhanced attachment and growth of A172 (human glioblastoma multiforme), 5H-SY5Y (human neuroblastoma) and PC-12 (rat pheochromocytoma)[275]. Therefore, sulfated biomimetic coatings can be used to modulate cellular responses through controlled growth factor binding or delivery as well as for biosensing applications.

4.C.4 Chitosan, Alginate, Agarose—As briefly reviewed in earlier sections, **chitosan** is a linear polysaccharide found in crustaceans and shellfish and serves as a versatile biopolymer which can be processed into various form factors, such as hydrogels, scaffolds, beads, and membranes[276]. Several studies have demonstrated chitosan's use in neural tissue engineering, showing its benefits towards promoting cell adhesion, interaction, survival, and neurite outgrowth[277,278]. Chitosan has been further combined with other materials to enhance the biocompatibility, electrical conductivity, and overall performance of the scaffold for neural tissue engineering applications. One study combined the biocompatible chitosan/gelatin porous hydrogel scaffolds with electrically conductive PEDOT nanoparticles via *in situ* polymerization which promoted neuron-like rat pheochromocytoma (PC12) cell adhesion and proliferation[220,279]. Another study used chitosan nanoparticles to impart hydrophilicity and high surface area to an electrically conductive polypyrrole (PPy)-alginate (Alg) composite scaffold[280]. Inspired by peripheral nerve conduits composed of polycaprolactone (PCL), another study formulated 50:50 chitosan-PCL solution for electrospinning nanofibers. Subsequently, the surface of these fibers was modified with a microlayer of alginate hydrogel containing neurotrophin-3 (NT-3) and conjunctiva mesenchymal stem cells (CJMSCs) as a stem cell source. This biomimetic scaffold demonstrated increased expression of Nestin, MAP-2 and β-tubulin III genes indicating successful differentiation of stem cells into neuron-like cells[281] for spinal cord injury treatment.

Alginate is a popular naturally occurring anionic biopolymer used in neural tissue engineering applications due to its biocompatibility, low-toxicity and gelation characteristics[282]. Previously, alginate gels were shown to promote peripheral nerve regeneration after injury in rat and cat models[283,284]. In recent years, different types of fibers have been introduced into alginate-hydrogels to improve its mechanical, electrical, and surface properties to make it more suitable for driving stem cell differentiation for repair and regeneration. For instance, graphite nanofilaments functionalized with citric acid can be embedded into an alginate matrix to provide local conductive zones and increase its mechanical stability for nerve regeneration[285]. Alginate hydrogels can also be modified with magnetic PCL short fibers which contained superparamagnetic iron oxide nanoparticles. Upon application of external magnetic field, these fibers aligned and accelerated the neural differentiation of encapsulated mesenchymal stem cells as shown in Figure 6 (F)[13]. Another formulation of polyvinyl alcohol/sulfated alginate nanofibrous scaffold with 30

wt% alginate concentration also exhibited a desirable surface attachment for Schwann cells and human bone marrow mesenchymal cells[286]. Studies showed that a gelatin-alginate formulation can be 3D printed by itself[287] or further mixed with different concentrations of carbon nanofibers and printed into an electroconductive hydrogel with desirable electrical, mechanical, and biocompatible characteristics[288]. One study introduced cobalt in a alginate/waterborne polyurethane 3D porous scaffold and reported increased neurite outgrowth (PC12 cells) as well as a notable transition from pro-inflammatory microglia phenotype (M1) to anti-inflammatory (M2) phenotype (BV2 cells).[289]

Given the resemblance of agarose to the ECM, **agarose**-based constructs can be used to not only emulate the brain tissue but also as a substrate in combination with other key components such as ECM proteins, growth factors and stem cells for neural tissue engineering applications. Freeze-dried agarose nerve guidance scaffolds loaded with brain-derived neurotrophic factor (BDNF) have shown promise with promoting host axon growth and reduced fibrotic encapsulation[290]. More recently, human fibrin-agarose hydrogels cross-linked with 0.25% glutaraldehyde has shown promising structural and biochemical properties for use as a nerve conduit intraluminal filler, especially when loaded with mesenchymal stem cells, to repair 10-mm nerve gaps in rats[291,292]. Agarose-collagen based hydrogel microcolumns (Figure 6 (G–I))seeded with neurons on one end were demonstrated as conduits that can be implanted in the brain to establish bidirectional communication with the host tissue[18,293]. These strategies can inspire biohybrid multi-modal applications which combine electrical elements with biocompatible components to promote seamless integration with the tissue.

5. Challenges of Applying Carbohydrates & Innovative Approaches

Carbohydrates are ubiquitously present in mammalian cells as part of the glycocalyx layer which not only serves as a protective barrier but also mediates cellular recognition and signaling. In the nervous system, carbohydrates or glycans have been deemed critical for tissue development and maintenance and their dysfunction is linked to several disease pathologies[106,171,295]. A vast library of distinct glycan patterns is formed because of the highly dynamic post-translational process of glycosylation which varies across different tissues. Despite their importance in human biology, their structural heterogeneity and complex functions are largely understudied. The major challenge in studying glycans is the inability to manipulate the expression of glycan structures using the genetic code, unlike their protein counterparts. In addition, the rich glycan heterogeneity complicates the analytical challenge further.

Currently employed analytical methods can assess the glycan structure, specific glycosylation sites, and the general abundance of glycans. These methods generally include high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), mass spectrometry (MS), isoelectric focusing (IEF) and lectin-based microarray. A combination of these methods may be utilized to analyze free oligosaccharides, glycosaminoglycans, and the glycan components of glycoproteins, proteoglycans, and glycolipids (as shown in Figure 7). More comprehensive review of these techniques is discussed elsewhere[296,297].

Even with advancements in these tools, there are limitations in the analysis of glycans. For methods relying on analyzing free glycans, they must first be enzymatically or chemically released from the conjugate structure and successfully retrieved. For samples with low glycan abundance, the glycan concentration and structure may be altered in this process[5]. For analysis of glycoproteins, the peptide component can be used as a tag for determining N- or O-linked glycosylation, after undergoing proteolytic cleavage to generate glycosylated peptide moieties. However, this approach can be challenging for studying highly glycosylated proteins which can generate a high number of heterogeneous moieties that may have limited accessibility for the MS analysis[298]. Another complication is that glycans are generally found attached to one specific glycosylation site on the protein and different N-glycosylation sites on one protein may have different glycan patterns. This poses a difficulty in analyzing glycoproteins using MS techniques where the sample may be contaminated with non-glycosylated peptides. Another bottleneck is the data analysis of complex structures originating from heterogeneous samples[298].

Novel chemical strategies to synthesize mimetic glycans have demonstrated to be an innovative approach in investigating the underlying glycan functions. Specifically, cell surface glycans can be metabolically engineered either by eliminating specific structural moieties by inhibiting the glycan synthesis or by incorporating chemically synthesized unnatural monosaccharide into the glycocalyx of the cells. In this way, synthetic carbohydrate probes can aid the mapping of glycan receptors in the nervous system by imaging or tagging them. The glycocalyx of the cells itself can be remodeled by inserting synthetic nanoscale glycan epitopes in a predefined spatial manner with minimal perturbations to the existing microenvironment[299]. Godula et al. synthesized sulfated heparan sulfate mimetics with affinity towards FGF-2 growth factors and subsequently introduced it in the plasma membranes of mouse embryonic cells deficient in HS biosynthesis[300]. Due to this deficiency, the cells are unable to signal via FGF2 which restricts them to an undifferentiated state. The addition of the HS mimetic then rescues the FGF2 signaling and induces neural differentiation. This approach has immense potential for facilitating neural tissue repair after injury. Hsieh-Wilson et al. conjugated chondroitin sulfate polysaccharides with liposomes for uptake by cortical neuronal membranes and discovered enhancement in growth factor binding in a sulfate dependent manner which ultimately promoted neural outgrowth[301].

Another important application of these synthetic glycomimetic is the conjugation with neuroactive molecules for delivery across the BBB to investigate the glycan environment, especially in diseased states. While biorthogonal chemistries have paved the way for live imaging of glycans in zebra fish and mice through metabolic expression of abiotic azido groups[302], expression of these molecules in the brain has been limited due to the impermeability of the BBB. Shahjahan et al. engineered carbohydrate-neuroactive molecules which crossed the BBB and modulated polysialic acids on neural cell adhesion molecules, thus changing the glycocalyx of these cells[303]. This proof-of-concept study demonstrated a non-invasive tool for investigating brain glycosylation as well as a route for drug delivery targeting specific glycan epitopes.

6. Future Directions

Advancements in material science, electrical engineering, and biomedical engineering have enabled the development of effective neural implants for restoration of lost function. Advanced techniques for live imaging of cellular response to these implants have also provided valuable insights into the dynamic changes that occur in the host tissue after implantation. This information can certainly guide the design and engineering of the next generation of neural implants. However, significant work is still required to design and engineer the ideal implant.

Novel biomaterials can help to bridge the gap between abiotic device interface and the biotic environment. Carbohydrates have been overlooked as biomaterial candidates for neural engineering applications, possibly due to the limited tools available for studying their heterogenous structures and complex function in the first place. They carry immense potential for incorporation at the interface to actively modulate the neighboring cell types without compromising the functional integrity of the device. Future work could entail the incorporation of a library of synthetic glycan mimetics, functionalized on the implant surface, which could target specific cell types and growth factors to modulate the microenvironment around the implant for favorable outcomes. For neural interface devices that utilize 3D scaffold to incorporate cells and/or promote neural regeneration on to the devices, the scaffold could harness the power of these biomimetic molecules to precisely control the architecture of the host tissue to drive repair after injury. While the structural heterogeneity of glycans may seem daunting to work with, with the right tools, their specific bioactivity can be exploited for developing the next class of neural implants.

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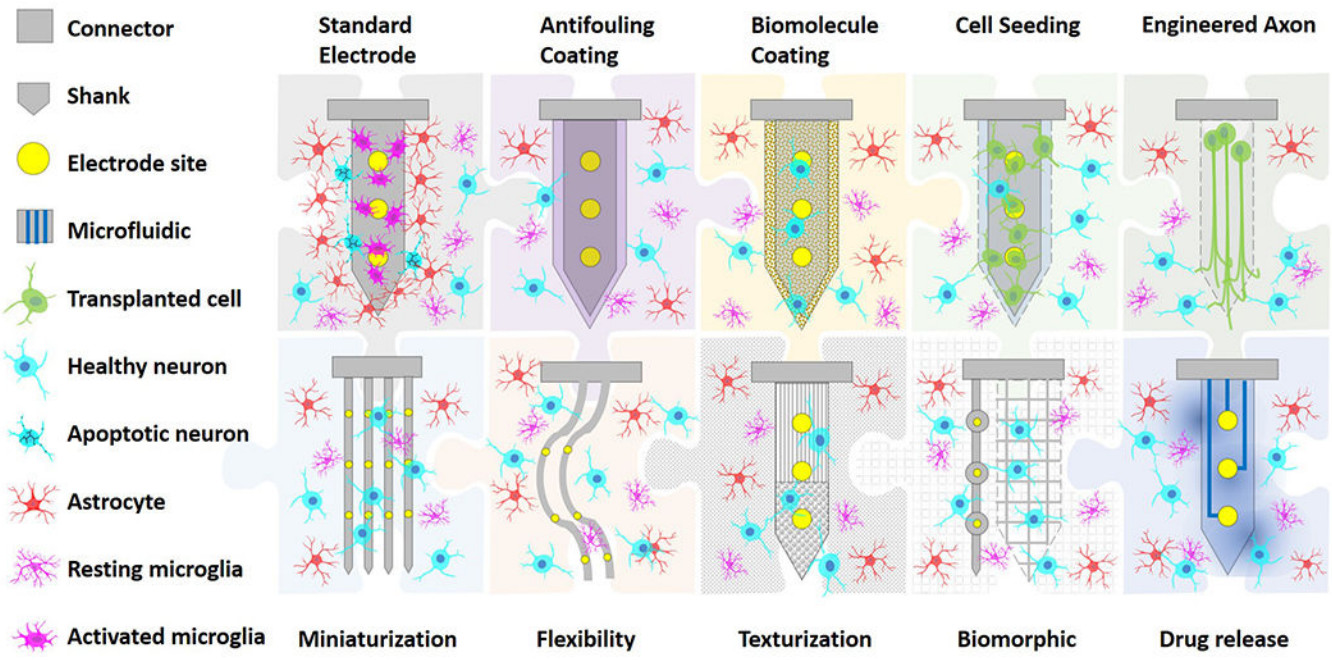

Figure 1:

Summary of design strategies that can reduce the host foreign body response to the implant. These approaches span from modifying the device factors such as shape, size, and surface chemistry to incorporating bioactive elements such as anti-fouling coatings, drug-delivery, biomimetic morphology and attachment of cells and tissues to the surface. These strategies used alone or in combination can promote seamless device-tissue integration. Adapted from Shi et al. with Creative Commons license[1].

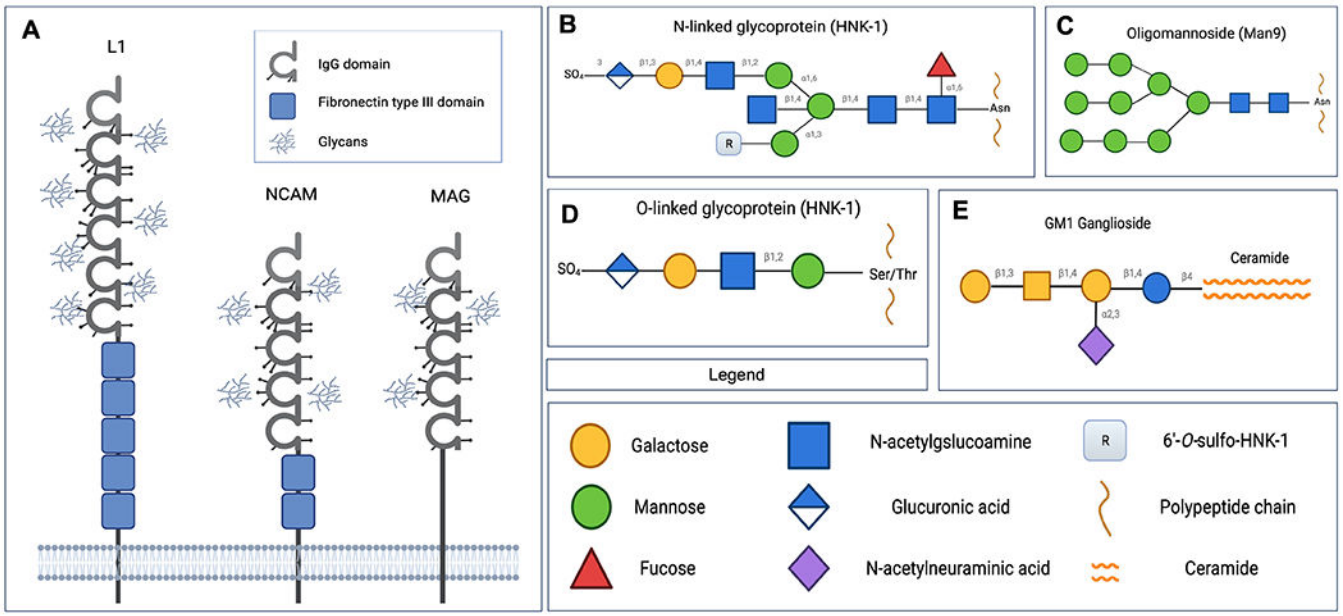

Figure 2:

Schematic showing glycan structure for representative glycoproteins and glycolipids present in the central nervous system. A) Examples of transmembrane glycoproteins anchored on the cell surface involved in various important cell-cell interactions via its glycan chains. Cell adhesion molecules L1 and neural cell adhesion molecule (NCAM) are structurally comprised of extracellular IgG domains and fibronectin type III (FNIII) domains, while myelin-associated glycoprotein (MAG) is comprised of only IgG domains. IgG domains are decorated with glycan structures post-translation. Adapted from Walsh et al[3]. B) Structure of human natural killer glycan (HNK-1) which is linked to the protein core via the nitrogen atom on the Asparagine (Asn) residue (called N-linked). C) Structure of oligomannoside mannose 9 which are present on N-linked glycoproteins. D) Structure of HNK-1 glycan attached to the protein core via the oxygen atom of either a Serine (Ser) or Threonine (Thr) protein. E) Structure of GM1 ganglioside glycan which is attached to a ceramide lipid. Adapted from Kleene et al[6]. Created with Biorender.com

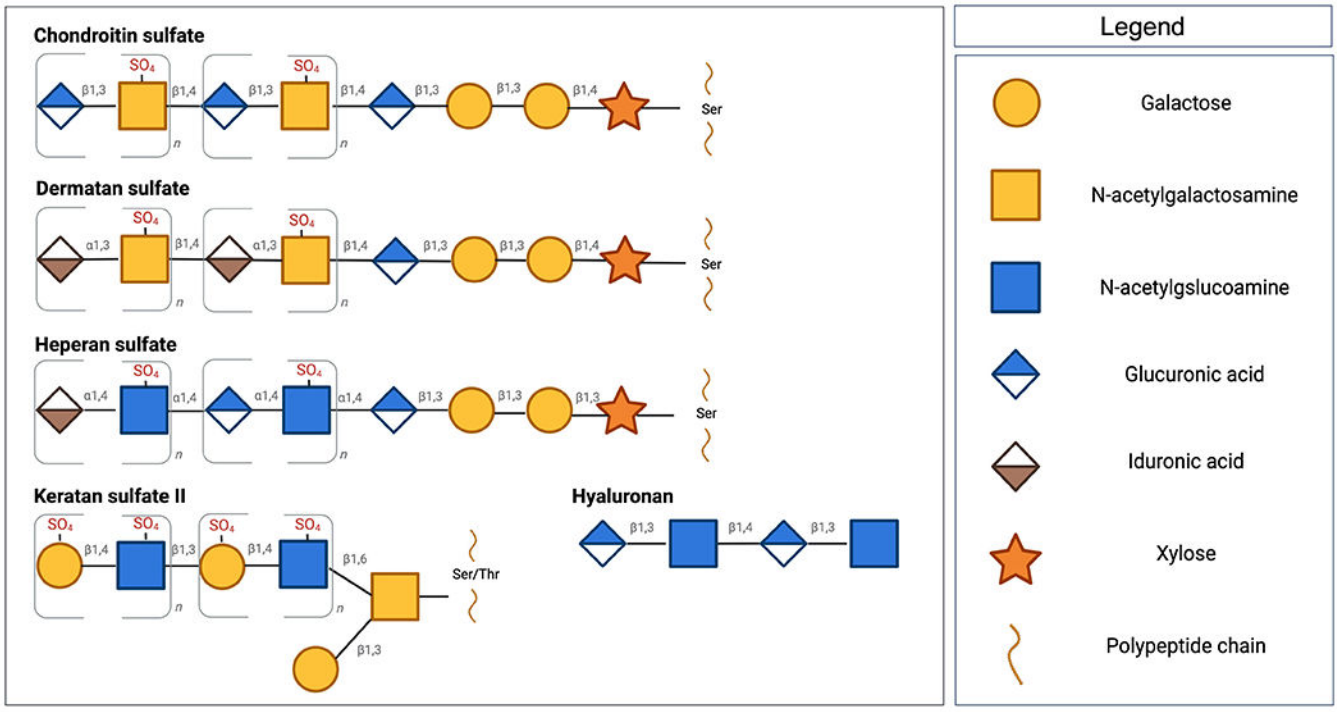

Figure 3:

Schematic showing the 5 main types of glycosaminoglycans (GAGs) which are composed of distinct combinations of repeating disaccharide units. The glycans carry a negative charge imparted by the sulfate groups attached to the disaccharide units. Except for hyaluronan, the glycans are attached to a protein core via a tetrasaccharide component comprised of glucuronic acid, galactose, and xylose. Adapted from Kleene et al[6]. Created with Biorender.com

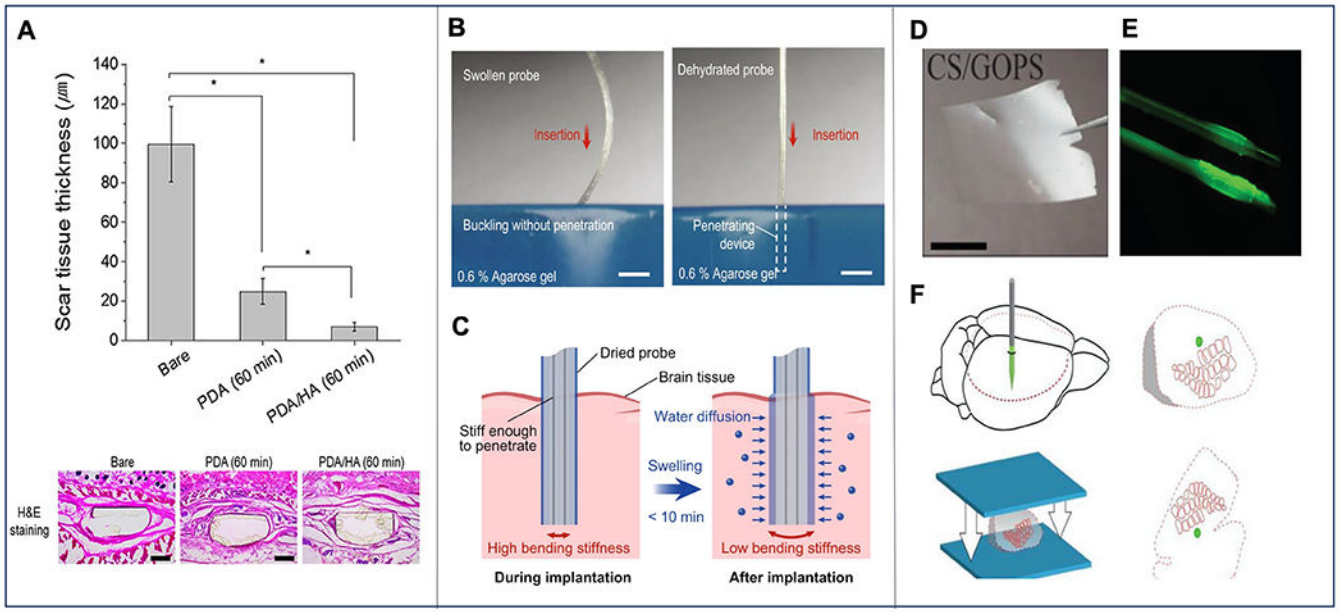

Figure 4:

Representative images showing examples of neural electrode surface modifications. A) Hyaluronic acid (HA) was co-deposited with polydopamine (PDA) which imparted hydrophilic property to the surface and demonstrated significant resistance to non-specific protein adsorption with no significant increase in impedance. Following a four-week subcutaneous implantation, PDA/HA modified electrodes revealed significant attenuation of scar tissue formation as compared to bare controls. Asterisks indicate statistical significance ($p<0.05$). Scale bars: 200 μm. Adapted from Kim et al[4] with Creative Commons License. B) A hybrid multifunctional probe composed of microscale polymer-based fibers encapsulated within a soft alginate-based hydrogel matrix was developed. These probes are capable of long-term neural recording, delivery of light and chemicals in the mouse brain. (Left) Image is showing the insertion of hybrid probe with fully swollen (left) and dehydrated (right) hydrogel matrix into the phantom brain (0.6% agarose) at the speed of 1 mm s^{-1}. Scale bar: 1 mm. C) Illustration of the adaptive bending stiffness of the hybrid hydrogel probes. When dehydrated, the probe exhibits sufficient bending stiffness for insertion. Following implantation, probes swells as it absorbs water from surrounding tissue while still exhibiting a lower bending stiffness, as compared to stainless steel fibers. Adapted from Park et al[12] with Creative Commons license. D) Chitosan based freestanding, stable and mechanically robust film before immersion in solution. Scale bar: 1cm. E) Fluorescent microscopy showing chitosan coated tungsten wires for recording. F) schematic showing CS-based brain marking, cortical flattening and slicing procedures. Adapted from Rauhala et al[15] with Creative Commons License.

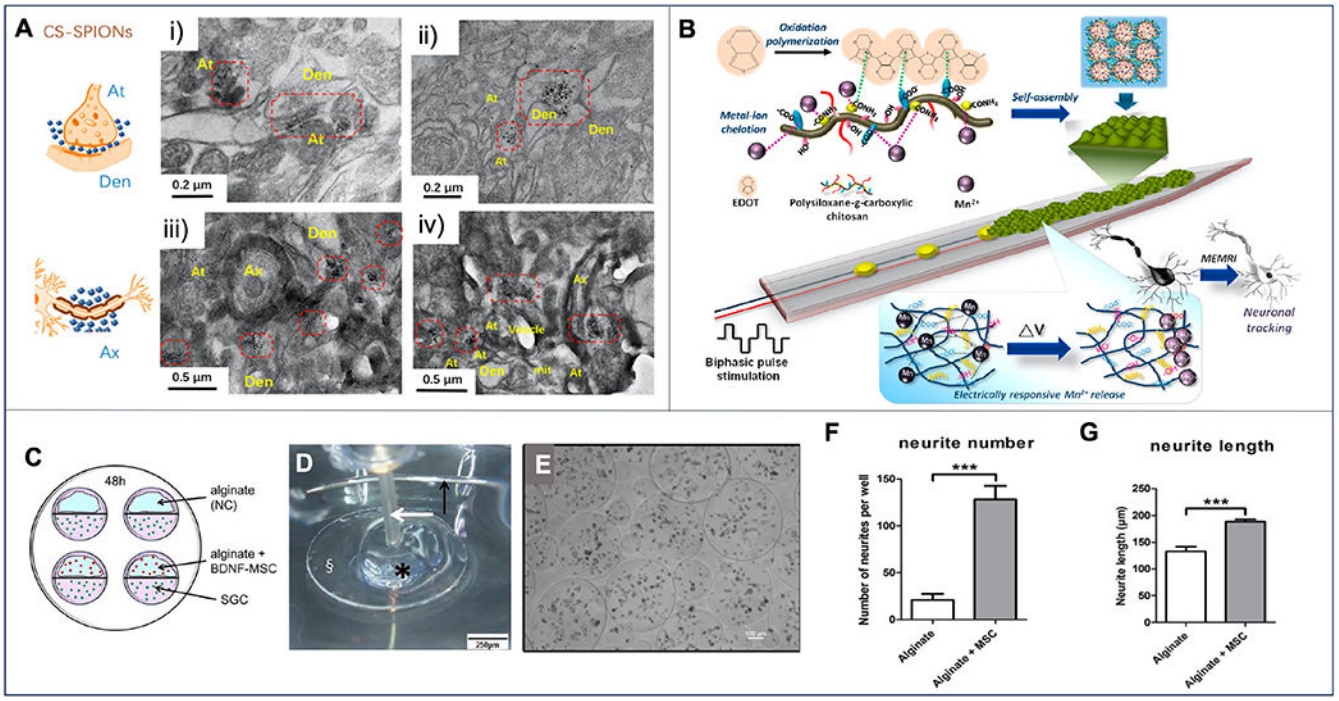

Figure 5:

Representative images highlighting drug-delivery approaches utilizing carbohydrate materials with applications for neural engineering. A) Superparamagnetic iron oxide nanoparticles (SPIONs) were functionalized with chondroitin sulfate (CS) and injected into the substantia nigra of rats. Transmission electron microscopy (TEM) showed that CS-SPIONS demonstrated lower endocytosis and were found in the intracellular spaces of neuronal cell bodies and between the synapses. i)-iv) CS-SPIONs highlighted in red rectangles found near the axons (Ax), axon terminal (At), dendrite (Den), mitochondrion (mit). Adapted from Nie et al[2] with Creative Commons License. B) Schematic of neural interface comprised of amphiphilic chitosan modified poly (3,4-ethylenedioxythiophene) (PMSDT) nanogel which enables metal-ligand bonding with Mn2+ ions, allowing the system to locally release Mn2+ ions by electrical stimulation and achieve real-time high-resolution manganese-enhanced magnetic resonance imaging (MEMRI). Adapted from Huang et al[10] with Creative Commons License. C). Ultra-high viscous alginate was used to encapsulate mesenchymal cells (MSC) from recipient's immune system which locally release brain-derived neurotrophic factors (BDNF) to improve the cochlear implant outcome. MSCs were further genetically modified to overexpress BDNF. A) Experimental set up for adjacent culture of alginate with BDNF expressing MSC (red dots) and spiral ganglion cells (SGC) (green dots), where alginate alone represents a negative control (NC) Petri dish with four internal rings. Internal wells are divided into halves separated by a liquid blocker (black line). Medium (pink) connected both cell compartments at the center. D) Experimental setup for electrical stimulation where cover glass (§) with polymerized alginate-MSC mixture (*) is placed in a petri dish. The active electrode (white arrow) was then placed on the alginate-MSC mixture, and an annular electrode served as ground (black arrow). The dish was filled up with MSC culture medium which is not shown

in the image. Scale bar: 250μm. E) Phase contrast image of encapsulated MSCs which was shown to be comparable to encapsulation at Day 0 (data not shown). Scale bar: 100μm. F) Adjacent cultivation of SGN with alginate encapsulated, BDNF producing MSCs significantly increased the number of regenerated neurites and their measured length. Data is presented as mean with standard error of mean (S.E.M.) Asterisks indicating significance between groups (**$p<0.01$, ***$p<0.001$). Adapted from Schwieger et al[16] with Creative Commons License.

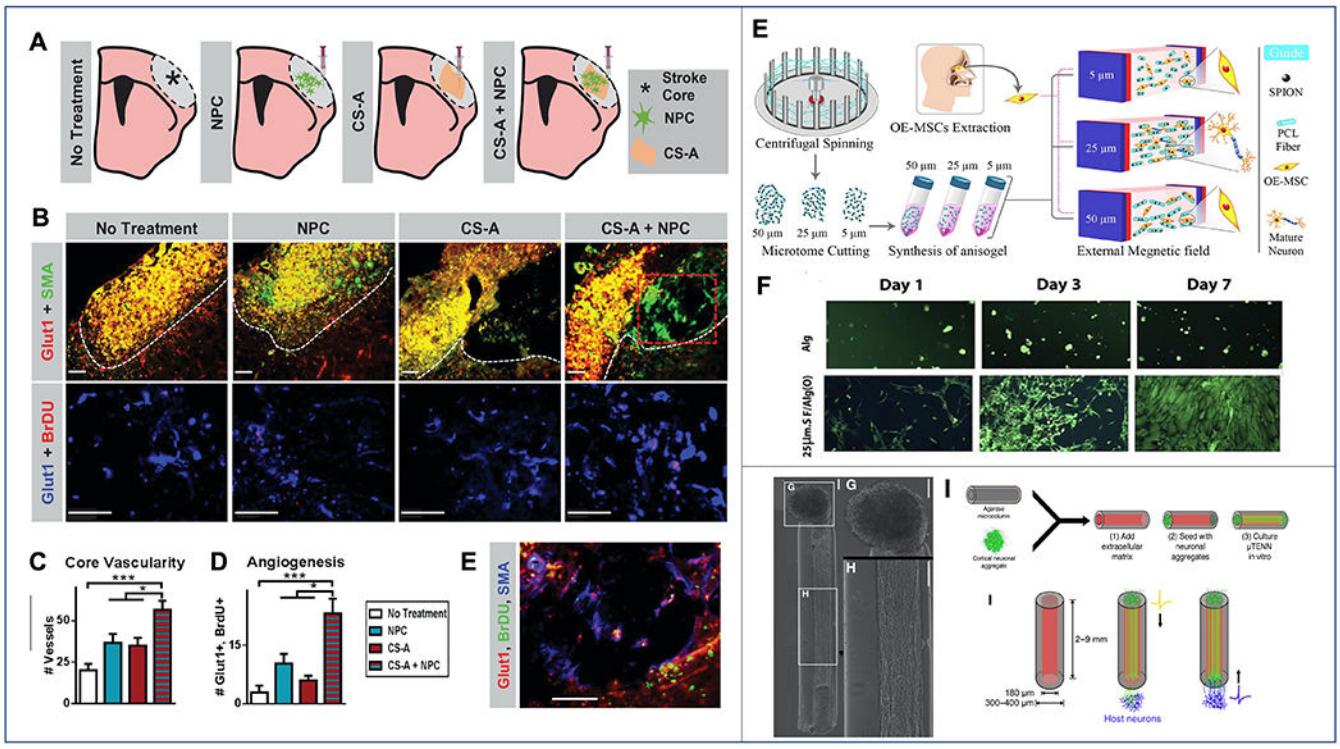

Figure 6:

Representative figures demonstrating the use of carbohydrate-based biomaterials for neural tissue engineering. A) Chondroitin sulfate-A (CS-A) hydrogel was embedded with neural progenitor cells (NPS) and transplanted into the infarct core in a mouse sensorimotor cortex mini-stroke model. Schematic in A) shows the treatment groups following stroke in mice, which include no treatment (sham injection), NPC alone, CS-A alone, and CS-A encapsulated NPC conditions. B) Top row shows the fluorescent images of Glut1 (brain endothelial cell marker) and SMA (new muscular arteries marker) staining illustrating vessels in the necrotic stroke core across four treatment groups. Core region indicated by the white dotted line. Area visualized in red dotted line is shown in E). Bottom row in B) shows fluorescent images of Glut1 and BrdU staining (proliferation marker) indicating formation of new endothelial cells across treatments. C) Quantification of the vascularity (# Glut1+ vessels) within the stroke core region. D) Quantification of angiogenesis (Glut1/BrDU co-labeled endothelium). E) Representative fluorescent image showing muscular artery formation (Glut1, SMA, BrdU) in the CS-A + NPC treatment group. Scale: 50 μm. *:$p < 0.05$, **:$p < 0.005$, and ***: $p < 0.0005$, respectively (1-way ANOVA, Tukey's post-hoc). Adapted from McCrary et al[9] with Creative Commons license. E) Schematic illustrates the general process of developing an injectable alginate hydrogel which is comprised of magnetic polycaprolactone (PCL) short nanofibers containing superparamagnetic iron oxide nanoparticles (SPIONs). The hydrogel with magnetically aligned fibers are embedded with olfactory ecto-mesenchymal stem cells (OE-MSCs) which showed cell viability after 7 days. F) Fluorescent images of live/dead assay showing the morphology of OE-MSCs in hybrid hydrogels cultivated for 1, 3 and 7 days. Top row shows the control alginate conditions and the bottom row shows the 25 μm magnetic short fibers with alginate condition. Live cells

were stained with FDA (green) and dead cells with PI (red). Adapted from Ghaderinejad et al[13] with permission. G-I) Representative images showing implantable “living electrodes” comprised of soft agarose-based hydrogel cylinder encapsulating long bundles of cortical neuronal axons. This micro tissue engineered neural network (μTENNs) can serve as conduits with transplantable input/output channels for optogenetics. G) Enlarged region showing discrete regions of cell bodies and neurite projections H). Scale bars in G) and H) are 100 μm. I) Schematic showing the agarose microcolumn (gray) filled with extracellular collagen-laminn matrix. Neuronal aggregates (green) are subsequently placed in the microcolumn terminal and grown in vitro. Adapted from Adewole et al[18] with Creative Commons license.

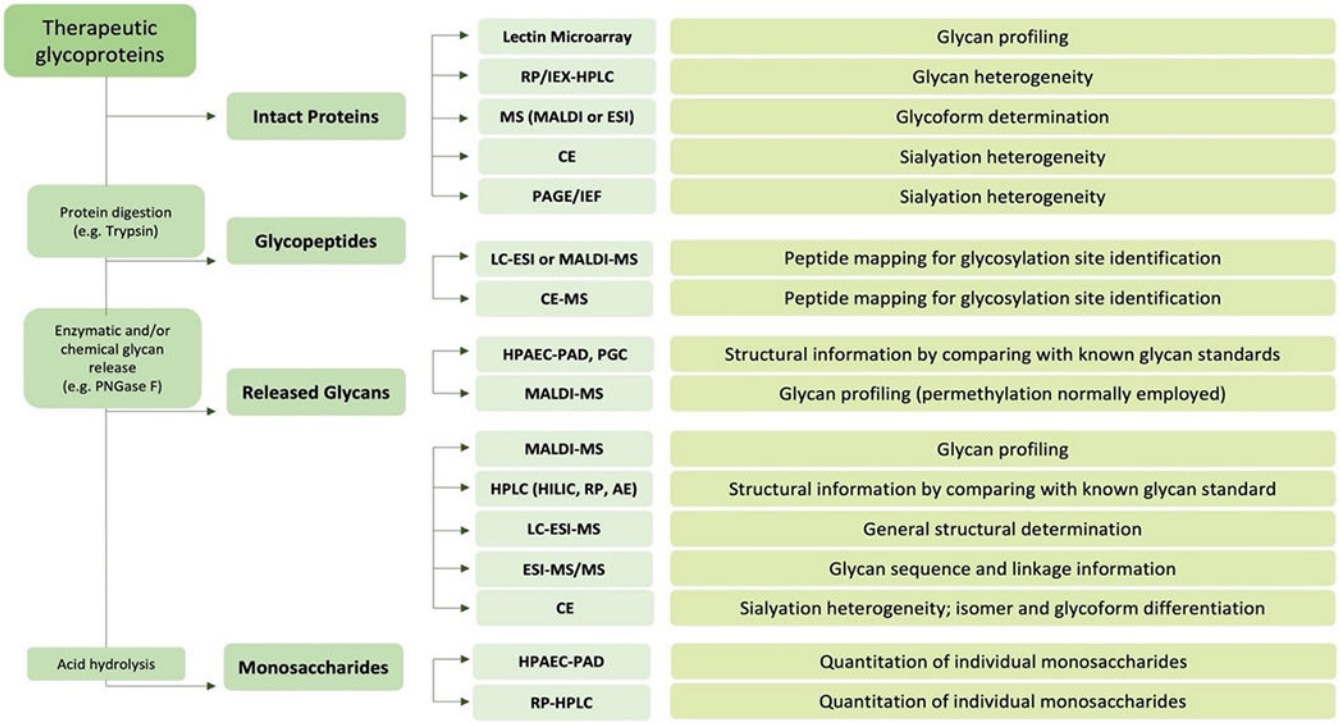

Figure 7:

Schematic overview of analytics assessing glycans. There are four main methods for glycan analysis based on their applications for analyzing intact proteins, glycopeptides, released glycans or monosaccharides. These methods can be used in combination to determine glycan profiles, structures, and heterogeneity along with glycosylation sites and the content for specific glycans. Adapted from Zhang et al[5] with Creative Commons license.

Table 1:

Summary of polysaccharide-based materials for neural electrode surface modification applications

Polysaccharides	Materials for Neural Electrode Surface Modification
Chondroitin sulfate (CS)	Poly(3,4-ethylenedioxythiophene) (PEDOT)-CS coatings[208–210]
Hyaluronic acid (HA)	PEDOT-HA coating[210]
	Polypyrrole (PPy)-HA coating[211]
	Multi-walled carbon nanotube (MWCNT)-HA nanofibers[212]
	PEDOT-HA/poly(L-lactic acid) (PPLA) composite film[213]
Chitosan	Chitosan coating[15]
	Chitosan-based conductive hydrogels[224,225]
	Chitosan-based glucose sensor encapsulation[225]
Alginate	Alginate conductive hydrogels[12,228,229]
Agarose	*In vitro* agarose brain phantoms[230]
	CNT-agarose hydrogel composite[231]

Table 2:

Summary of polysaccharide-based materials for neural drug-delivery applications

Polysaccharide	Materials for Neural Drug Delivery
Chondroitin sulfate (CS)	CS-conjugated nanoparticles[2,237] and CS-based stabilizing agents for metallic nanoparticles[238]
	Modified CS sidechains for hydrophobic drug encapsulation[239]
	Exogeneous CS-coating for DNA complexes[240]
Hyaluronic acid (HA)	HA-conjugated liposomes and HA-based lipid nano emulsions[237,242,243]
	MWCNT-HA[244]
Chitosan	3,4-Ethylenedioxyphene (EDOT)-Chitosan nanogel composite[249]
	Chitosan hydrogels[10,250]
Alginate	Alginate hydrogels for drug encapsulation[16,221,254]
Agarose	Agarose gel matrix for drug encapsulation[221,255]

Table 3:

Summary of polysaccharide-based materials for neural tissue engineering applications

Polysaccharide	Materials for Neural Tissue Engineering
Chondroitin sulfate (CS)	CS-matrices for encapsulation of growth factors or stem cells[262–265,268,294]
	CS-coating for neurite growth promotion[184]
Hyaluronic acid (HA)	HA-based hydrogels[88,268]
	Polycaprolactone-HA-based electrospun scaffolds[269,270]
Heparan sulfate (HS)	HS-based porous scaffold[272]
	HS-mimics based hydrogels[274,275]
Chitosan	Chitosan based conductive scaffolds[220,279,280]
	Chitosan-based hydrogel for encapsulation of biological molecules[281]
Alginate	Fibrous alginate-based hydrogels[13,285,286]
	3D printed alginate-based scaffolds[287,288]
	Biomimetic alginate-based scaffolds[281,289]
Agarose	Agarose-based hydrogel scaffolds for nerve guidance[18,291–293]