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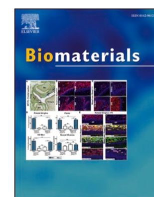

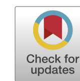

Glycosaminoglycans' for brain health: Harnessing glycosaminoglycan based biomaterials for treating central nervous system diseases and *in-vitro* modeling

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ABSTRACT

Dysfunction of the central nervous system (CNS) following traumatic brain injuries (TBI), spinal cord injuries (SCI), or strokes remains challenging to address using existing medications and cell-based therapies. Although therapeutic cell administration, such as stem cells and neuronal progenitor cells (NPCs), have shown promise in regenerative properties, they have failed to provide substantial benefits. However, the development of living cortical tissue engineered grafts, created by encapsulating these cells within an extracellular matrix (ECM) mimetic hydrogel scaffold, presents a promising functional replacement for damaged cortex in cases of stroke, SCI, and TBI. These grafts facilitate neural network repair and regeneration following CNS injuries. Given that natural glycosaminoglycans (GAGs) are a major constituent of the CNS, GAG-based hydrogels hold potential for the next generation of CNS healing therapies and *in vitro* modeling of CNS diseases. Brain-specific GAGs not only offer structural and biochemical signaling support to encapsulated neural cells but also modulate the inflammatory response in lesioned brain tissue, facilitating host integration and regeneration. This review briefly discusses different roles of GAGs and their related proteoglycan counterparts in healthy and diseases brain and explores current trends and advancements in GAG-based biomaterials for treating CNS injuries and modeling diseases. Additionally, it examines injectable, 3D bioprintable, and conductive GAG-based scaffolds, highlighting their clinical potential for *in vitro* modeling of patient-specific neural dysfunction and their ability to enhance CNS regeneration and repair following CNS injury in vivo.

1. Introduction

1.1. Brain ECM and glycosaminoglycans

Extracellular matrix (ECM) is the complex network of macromolecules such as proteins, glycoproteins and glycosaminoglycans that provide the structural integrity and regulate several cellular processes. Unlike other tissues, brain ECM is mostly devoid of fibrous proteins such as collagen, elastin, and laminin, and is filled with amorphous gel derived from glycosaminoglycans (GAGs) like hyaluronan (HA), chondroitin sulfates (CS), and glycoproteins. Fibrous proteins in the brain are mainly found in basal lamina around the blood vessels and near the surface of the brain tissue [1]. Further brain matrix molecules found in basement membranes throughout the brain like heparan sulfate (HS), laminins, collagens, and fibronectin are produced by neurons, glia, and endothelial cells and help secure the brain by maintaining the blood-brain barrier [2]. GAGs are linear polysaccharides with well-defined disaccharide repeating units composed of N-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), or galactose (Gal), with glucuronic acid (GlcA) or iduronic acid (IdoA). They are ubiquitously present in the native extracellular matrix (ECM) throughout the body, in the intracellular milieu, and on the cell surface of all animal cells [3]. GAGs come in six major forms that differ in their sulfation degree, locations, and patterns. The six major GAG forms include CS, heparin (HP), heparan sulfate (HS), dermatan sulfate (DS), keratan sulfate (KS), and HA [3]. Among different ECM polymers, HA, CS and HS constitute the major component of the brain ECM [4].

Among these ECM polymers HA is the only GAG which is non-sulfated and are not covalently linked to any proteins. All other GAGs are sulfated, and exist as proteoglycans (PGs) that is, these polysaccharides are covalently conjugated to a protein core. The CS, DS, HP, and HS forms the PGs by *O*-linked covalent coupling of GAGs to the serine residues via a common tetrasaccharide, xylose-galactose- galactose-glucuronic acid. KS on the other hand is N-linked to asparagine residues via *N*-acetylglucosamine [5]. These proteoglycans actively regulate nervous tissue development by promoting or inhibiting neurite outgrowth, maturation/differentiation of stem cells or progenitor cells and regulate synaptogenesis and migration. The vast majority of the brain ECM is composed of PG structures with hyaluronan backbones with branching chondroitin sulfate proteoglycans (CSPGs) of the

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lectican family along with tenascins [6]. These ECM molecules are produced by all brain cell types including neurons, astrocytes, oligodendrocytes, and microglia, and thus are present in all brain structures. CS is the most prevalent and most studied GAG in the brain ECM. CS guides axonal pathfinding during development and growth, and later after development, CS is widely found in perineuronal nets and has been shown to play a role in limiting synaptic plasticity [7–9]. The sulfation pattern especially in the case of HS and CS, also play an important role in regulating cellular processes by altering the binding to growth factors and proteins, thus display distinct biological functions [10]. Additionally, HS could display either pro-angiogenic activity by binding with VEGF or FGF-2 or could display anti-angiogenic activity by binding with endostatin [11].

The diversity and structural differences in sulfation patterns in GAGs derives from their dynamic enzymatically regulated biosynthesis process that does not follow the usual DNA-RNA-Protein production pathways. The biosynthesis location and processing within the cell further varies between GAG types. For HP, HS, CS, and DS, biosynthesis begins in the late endoplasmic reticulum and/or the cis-Golgi compartment and the production of the type of GAG is determined by molecular composition of the secondary saccharide that is added to the initiating tetrasaccharide linker. For HA, however, the biosynthesis process occurs mainly in the cell membrane and does not require the linkage to an initiation protein in a reaction catalyzed by hyaluronan synthase 1, 2, or 3 enzymes [12].

1.2. Role and function of GAGs in brain development

In the brain, GAGs help guide development and provide the structural support for healthy homeostatic brain tissue [12,13]. GAGs also impart guidance for damage response and neuroplasticity of the CNS [14, p. 6]. The structure and function of GAG chains can vary temporally and spatially in the brain during development with tight regulations guiding GAG assembly and biosynthesis in cells. Aberrations in the assembly and structure of GAGs can have an effect on the development of disease states, and GAG states can be altered in response to injury or during healing [12]. Brain development can be guided by the production of GAGs by various cell types as well. HA, for example, can bind ECM proteins such as heavy chains shifted from inter-α-inhibitor at sites of inflammation to form stabilizing complexes to heal the CNS. The binding of HA to ECM proteins allows for the formation of Perineuronal Nets (PNNs) on the surface of neurons from HA ternary complexes. PNNs help protect and stabilize neurons from disease and external harm, and they also take part in controlling neuroplasticity both pre and post injury or neurodegeneration throughout life [15]. Fig. 1 illustrates the various functions GAGs impart within the brain in both healthy and diseased brain regions.

Several of these brain ECM molecules undergo transformation following CNS trauma and in CNS disease states. During development, both heparan sulfate proteoglycans (HSPGs) and CSPGs direct the vast majority of neuronal development in the CNS. HSPGs help localize growth factors and morphogens at specific sites through coordinated binding. CSPGs, on the other hand, restrict axonal growth and act as barrier molecules to guide neurons towards the correct shape and fidelity during development [7]. In dynamic coordination together, these GAGs and their larger PG structures provide instrumental information to help the developing brain. GAG structure, their heterogeneous sulfation patterns, and their biosynthesis pathways during development are precisely regulated by cell type, cell cycle, and cell-cell communications through various cell surface proteins [13]. The varied collection of GAGs and sulfation patterns result in the creation of specific binding domains for ligands such as growth factors in time and space. According to previous studies, CS and HS can bind selectively to growth and transcription factors like FGF, Wnt, and Sonic Hedgehog [16,17]. The binding of growth factors to HS can also increase their half-life by sequestering them for later use, control diffuse release into surrounding microenvironment, act as coreceptors in signaling pathways, control the removal process through endocytosis, and ultimately protect them from degradation [18].

Studies show that during CNS development, HSPGs control axon guidance and neuronal development by encoding transcription factor distribution and growth factor signaling. CSPGs, on the other hand,

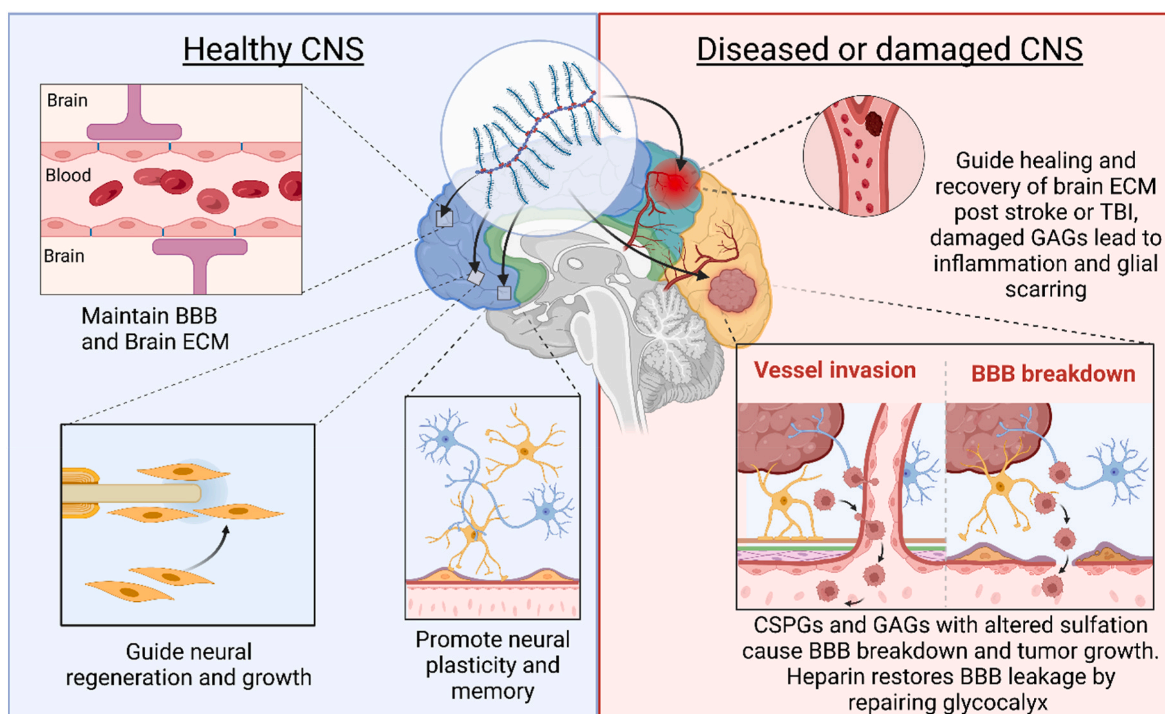

Fig. 1. Glycosaminoglycans play pivotal roles in maintaining healthy brain ECM functions. GAGs further provide cues for healing, guide regrowth after damage, and can provoke the establishment of brain disease states when altered in composition. Created with Biorender.com.

provide inhibitory cues to help with axonal pathfinding, guidance, and synapse formation [8,19,20]. During the course of development, the overall amount of CS in the CNS decreases and the sulfation patterns of CS changes, and previous studies have well documented the difference in CS sulfation pattern between the developing and mature CNS [7,13]. CSPGs and DS moreover influence stem cell proliferation and differentiation in CNS stem cell niches [21]. HA also provides critical function in the formation of the brain ECM during CNS development. HA, as a volume filling shock absorbing molecule, is produced by cells in the notochord and neural tube during development. HA can be found ubiquitously in the CNS on the cell surface of cells during development with the largest concentrations in densely cell packed areas of the cerebellum ECM, cerebral grey and white matter, peri-ventricular germinal layer, and external granular layers of the cerebellum. HA generally remains attached to the neuronal cell surface by selectively binding to cell surface HA receptors like CD44, HA binding proteins like versican, and to HA synthases 1, 2, and 3 during biosynthesis. HA further interacts with numerous growth factors and cytokines in the brain ECM that are instrumental in brain development including TGF-beta, bone morphogenic protein superfamily members, interleukin-1 beta, tumor necrosis factor-alpha, epidermal growth factor (EGF), keratinocyte growth factor, along with various others [22].

Synapse formation is also largely guided by CSPGs and HSPGs through GAG-PG receptors, specifically receptors of the leukocyte common antigen-related (LAR) protein tyrosine phosphatases family (RPTP). These receptors provide adhesion sites for synaptic molecules to organize synaptic development. This unique coordination between GAGs and the formation of complex PG structures directs many important physiological processes during development. GAGs have further been shown to interact, sequester, and modulate chemokines, cytokines, ECM proteins, enzymes, and inhibitors. Research remains limited on the effect of other GAG types on CNS development, but early research indicates all GAG types including KS and DS in addition to the previously discussed GAGs may all contribute to fully functioning brain ECM and CNS development [23].

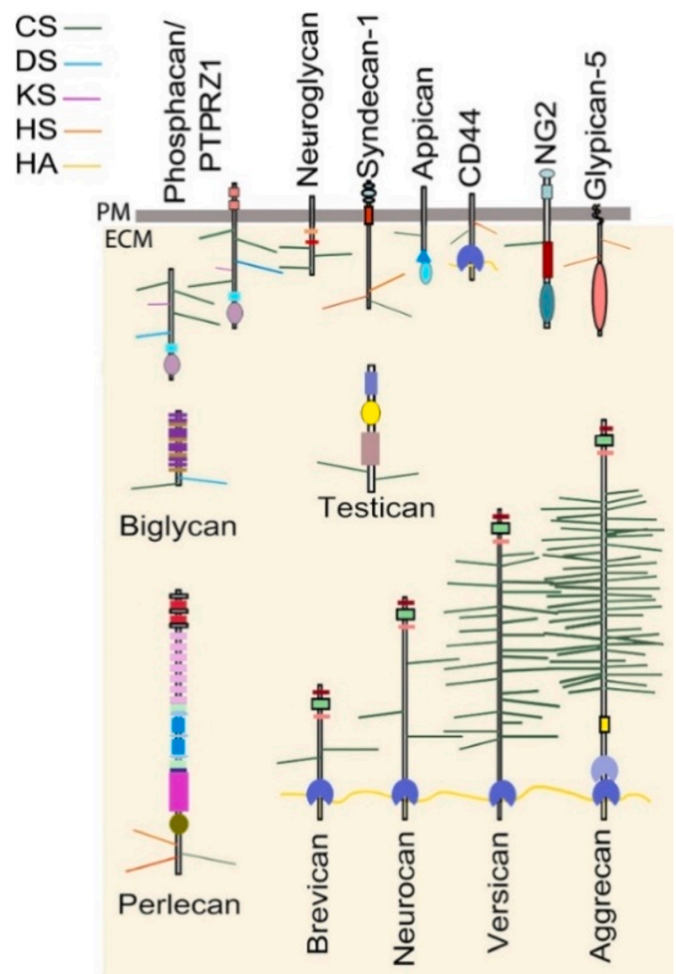

Fig. 2. Extracellular and membrane-bound proteoglycan families found in the central nervous system. Adapted from Ref. [5] with CCC Rights Link permission (License Number: 5678811486063).

1.3. Proteoglycans in the CNS

1.3.1. PGs structure

As briefly mentioned prior, the neural ECM is largely composed of GAG linked PGs. These GAG-PGs are composed of a core protein polypeptide back-bone and one or more linear chains of GAGs. These GAGs are linked to core proteins through different mechanisms, such as O-linked and N-linked glycosylation. Over 30 core proteins have been characterized and classified into different gene families and functional groups. The lectican family includes common extracellular matrix constituents, such as aggrecan, versican, neurocan, and brevican. Glypican family proteins bind to the cell surface through a glycophosphatidylinositol (GPI) anchor, while transmembrane family proteins integrate into the cell membrane, including syndecans, neuroglycan, appicans, and NG-2 [5]. The different GAG chains and their associated core proteins play important roles in various physiological processes in the brain and CNS. In this section, we explore the diversity and function of GAG-PGs and their roles in the CNS and brain ECM. Fig. 2 illuminates the PG families present in the CNS.

1.3.2. Neural PGs

1.3.2.1. Large lectican neural PGs.

i) *Aggrecan* is a core protein attached to the KS and CS side chains in the CNS and PNS. These components provide unique functional properties to the ECM and establish ionic gradients and micro-compartments essential for neural cell populations. Aggrecan's ability to absorb water and form macro-aggregates with HA gives it space-filling and matrix-stabilizing properties. The GAGs associated with aggrecan have neurite outgrowth-inhibitory activity, and their sulfation position and density are important for tissue development and neuroprotection. Generally, the aggrecan plays a dominant role in neuronal cell migration and astrocyte differentiation, neuroprotection, synapse formation and synaptic plasticity, memory, and learning [24].

ii) *Versican* is a large ECM PG, 400 kDa core protein, with CS and DS side chains. Versican comprises of five isoforms due to alternative splicing of primary exons, namely V0, V1, V2, V3, and V4 [25]. Versican interacts with many ECM and cell proteins, such as HA, HNK-1-substituted cell adhesion proteins and glycolipids, collagen type I, fibrillin, fibronectin, CD44, P- and L-selectin, integrin β1, EGF-R, and TLR2 [26]. Thus, versican plays versatile structural and functional roles in brain, including CNS development, cell migration, maturation, and differentiation. Versican, V1 and V2 are highly expressed in the mature brain and are involved in neural differentiation and neuritogenesis, as well as angiogenesis. A matricryptic fragment of versican, versikine, is generated by ADAMTS cleavage leading to immunoreactivity and cellular apoptosis. Versican isoforms are differentially found in various brain tumors such as gliomas, medulloblastoma, schwannomas, neurofibromas, and meningiomas [27].

iii) *Neurocan* exists as a component of PNNs with a broad distribution in the CNS/PNS. Neurocan and plays a role in PNN formation, synaptic signaling regulation, neurite outgrowth inhibition, axonal regeneration, and neurodegenerative disorders. To be more specific, neurocan has been found to modulate neural migration, neurite outgrowth and axonal development through interactions with tenascin-R, Gpc-3, Sdc-1, and PTN [24,27,28].

iv) *Brevican,* as the smallest member of lectican family, plays a role in stabilizing synaptic connections and synaptogenesis, regulating neural plasticity and axonal growth, upregulating in glial scars, and neural cell behavior. Moreover, brevican can be cleaved to produce a N-terminal bioactive fragment called Brain-enriched hyaluronan-binding protein (BEHAB). This BEHAB protein is highly expressed in human gliomas and associated to progression of gliomas [29].

1.3.2.2. Non-lectican large neural PGs.

i) *Phosphacan*, an mRNA splices variant receptor protein tyrosine phosphatase -beta/zeta (RPTP-ζ/β), is a transmembrane CS/KS-PG that interacts with neural cell-adhesion molecules such as N-CAM, axonin-1, and TAG-1 and matrix protein like tenascin-C [16]. Phosphacan are highly expressed in nerve fiber–rich layers, such as cerebral and hippocampal neurons, astrocytes, and oligodendrocyte precursor cells (OPCs). Thus, it plays a key role in modulation of neural development, neural network formation, neuron-glia cross-talk, and axonal repair [30].

ii) *Perlecan* is a multi-domain HSPG found in basement membrane preserving the integrity of BBB. Perlecan is a multifunctional protein in neural tissue development, homeostasis, and diseases through interactions with various growth factor such as EFG, FGF, and VEGF. To be more specific, it has a main role in neural stem cell self-renewal and neurogenesis by regulating GF signaling, neural repair after ischemic stroke or CNS injury via modulating pericyte recruitment, and Alzheimer's disease (AD) and amyloid disorders [31].

iii) *Neuron-glial antigen 2 (NG2)* is a class I transmembrane CSPGs with 252 kDa core protein primarily expressed by OPCs. NG2 mainly correlates with pathological situation such as brain and spinal cord injury and neurodegenerative diseases. It repairs damages by proliferation and differentiation OPCs via activation of $\beta 1$ integrins, PDGF-AA, and FGF-2 signaling, and axonal regeneration through RPTPσ, RPTPζ, NgR, 1 and NCAM complex pathways [32,33].

iv) *Agrin* is a basement membrane multifunctional HPGS with 225 kDa core protein originally detected in the neuromuscular junction (NMJ) yet found in various tissues. In addition to formation and maintenance role of agrin in NMJ, it has a pivotal role in synaptogenesis induced by aggregation of acetylcholine receptors (AChRs) following by MuSK activation. Furthermore, agrin is implicated in CNS development particularly in axonal growth, BBB integrity via improving matrix adhesion at brain endothelium, and AD related to neurofibrillary tangles formation [34,35].

1.3.2.3. Small lectican neural PGs.

i) *Syndecan* is a transmembrane HS/CS-PGs with four subtypes occur in vertebrates, including Sdc1, Sdc2, Sdc3, and Sdc4, which are primarily found in epithelial tissues, fibroblasts, neuronal tissues, and multiple cell types, respectively. Sdcs regulate cell adhesion and intercellular signaling, impacting neural plasticity, axonal growth, neural development, and neurodegenerative diseases [36].

ii) *Glypicans* are HS/CS-PGs anchored by glycosylphosphatidylinositol (GPI) to the plasma membrane. They have an integral interaction with synapse-organizing proteins and act as ligands for LRRTMs, PTPσ, Nrxs and GPR158, regulating synapse formation and function. Therefore, they play a pivotal role in synapse development and signaling pathway. In addition, glypicans malfunction may cause neurodevelopmental and psychiatric disorders such as autism and schizophrenia [37].

iii) *Neuroglycan C (NGC)* is a 150 kDa transmembrane CSPGs containing an EGF-like extracellular domain. NGCs are highly expressed the striatum, hippocampus, amygdala, and cerebral cortex functioning in the modulation of synaptic plasticity induced through tyrosine kinase receptors of the ErbB family, neuritogenesis mediated via PI3K and PKC pathways [38,39].

iv) *Biglycan* is a CS/DS-PGs consisting of 45 kDa protein core generated by astrocytes and immune cells. Biglycan is involved a broad range of implication in promoting the survival of neocortical neurons, modulating neuroinflammation through TLR signaling, inducing microglial activation via TLR4/NF-κB signaling after subarachnoid hemorrhage, inhibiting neurite outgrowth of sensory neurons, upgrading the stability of neuromuscular synapses, and enhancing immunoreactivity in spinal cord and cerebrum [23,40].

v) *Appican* as a CSPG form of amyloid precursor protein (APP) provides a pivotal role in the development of AD. As an illustration, the expression of appican by astrocytes proposes functions in stimulation of neural cell adhesion and neurite outgrowth, leading to the development of pathological structures such as neuritic plaques and brain scars [41–43].

vi) *Testican* is CS/HS-PGs with multidomain protein core related to BM-40/SPARC/osteonectin families, which involves in cell-cell/matrix interactions and neural growth regulations. Testican-1 is associated with diverse function including suppression of cathepsin-L, inhibition of MMP activation and neurons attachment, co-aggregation with Plaques in AD migration, and promotion of axonal regeneration. In contrast, testican-2 abolishes the inhibition of matrix metaloprtoeases (MMPs), inhibits neurite extension, and modulates neuronal development. Testican-3, exclusively expressed by thalamic nuclei, contributes to the neuronal formation and maintenance [44].

vii) *Decorin* is a class I small leucine-rich CS/DS-PG with 42 kDa protein core highly expressed in neocortex, hippocampus, thalamus, myelinated fibers, and mesenchymal tissues. Decorin promotes of axonal regeneration by suppression inhibitory ligands and modulates neuroinflammation and neuroprotection by blocking TGF-$\beta 1$ and TNF-α signals. Moreover, it has a role in neurodegenerative diseases including AD via autophagy-lysosomal signaling and MS through aggregation of (PDGFR)β -positive cells [45,46].

1.3.3. PGs functions in the CNS

1.3.3.1. Migration. In the developing neocortex, pyramidal neurons initially take a multipolar shape in the ventricular zone, migrating randomly in subventricular and intermediate zones. Transitioning to a bipolar shape, they attach to radial glial fibers, migrating towards the marginal zone in an "inside-out" arrangement [47]. Inhibitory neurons

migrate tangentially, influenced by specific cortical layers, GAGs, and PGs [48]. Pleiotrophin-Phosphocan signaling promotes migration, while soluble CSPGs in the marginal zone inhibit neuronal migration [5]. Neuroglycan-C, linked to Börjeson-Forssman-Lehmann syndrome, binds pleiotrophin and midkine, contributing to radial neuronal migration. Syndecan-3 and syndecan-1, binding partners of pleiotrophin, impact cortical neuronal migration [49]. Cortical interneurons, originating from specific regions, migrate under chemo-attractive and chemo-repulsive influences regulated by CS/HS-PGs [7]. PGs in the marginal zone and subplate can act as either attractive or repulsive substrates based on bound proteins [49].

1.3.3.2. Synaptogenesis and synaptic plasticity. Neural synapses generate when neurotransmitters release into the synaptic cleft, binding to receptors on postsynaptic membranes. HSPGs like SDC2, CASK, synbindin, synectin, neurofibromin, and EphB2 are vital for postsynaptic process [50]. GPC4, a presynaptic partner of LRRTM4, binds to PTPRS, fostering the development of excitatory synapses. Astrocytes contribute by secreting GPC4 and GPC6, encouraging the clustering of glutamate receptors in retinal ganglion cells [51].

Additionally, CSPGs, key components of PNNs, play a role in stabilizing and maturing neural synapses. The degradation of PNNs, containing aggrecan, neurocan, versican, and phosphacan, has functional consequences affecting ocular dominance, fear memory resilience, and enhanced long-term recognition memory. CSPG receptors SEMA3, LAR, and NOGO, present in PNNs, emphasize the importance of PGs in axon guidance and the regulation of synaptic plasticity [5].

For instance, PNNs around parvalbumin positive interneurons control plasticity. The digestion or genetic attenuation of PNNs prevents and reverses critical period closure. During critical periods, notable changes in the sulfation patterns of CSPGs occur, impacting plasticity. Manipulating Chst3, such as overexpression or deletion, affects ocular dominance plasticity levels in mice, highlighting the dynamic role of CSPGs in synaptic processes [52].

1.3.3.3. Axonal guidance and pathfinding. GAGs have been shown to play a significant role in axon guidance and pathfinding in the development and maintenance of both the peripheral and central nervous systems. The major guidance cues from factors like netrins and slits bind and sequester to high affinity HSPGs [53]. In various experiments such as those involving cockroach embryos and the Xenopus optic system, the addition of exogenous heparan sulfate or the removal of heparan sulfate caused axon defasciculation and growth in wrong directions [34]. Furthermore, HSPGs can activate proteins such as APP, laminin, and FGF-2 which can also stimulate neurite outgrowth [34]. Transgenic mice lacking HS 2-O- or HS 6-O- sulfotransferase revealed dysfunction of axon guidance at the optic chiasm [49]. Additionally, studies have suggested that HS contributes to the pathfinding and sorting of retinal axons. The studies further illustrate that HS is critical for cell proliferation during brain development [54].

CSPGs are also associated with axon guidance during development, as they can act as barriers to axonal growth, directly impeding axonal growth and guidance. Specific sulfation patterns of CSPGs also affect axonal guidance, with CS sulfated at the 4-position acting as a negative guidance cue for growing axons. Knockdown of the CS biosynthetic enzyme 4-O-sulfotransferase enhances axonal growth [55]. Furthermore, CSPGs play a part in axon myelination with the expression of brevican during CNS development [56]. CSPGs moreover regulate cell proliferation, with CS polysaccharides promoting the proliferation of neural stem or progenitor cells [34,57].

1.3.3.4. Memory. Neural ECM and neural specific PGs are integral parts of memory, specifically impacting PNNs. The correlation between ECM and memory has been related to digestion of CSPGs with chondroitinase ABC and MMPs, leading to multiple types of memory and possible memory loss [52]. Deletions of either link protein Crtl1 or aggrecan, and neutralizing C4S with a blocking antibody (Cat316), have all been linked to enhanced memory acquisition or retention [58]. C6S levels in PNNs are drastically reduced during ageing, leading to memory loss. Restoring C6S and/or neutralizing C4S with Chst3, Chst11, and Cat316 antibody treatments, have been used to treat memory loss [14,52]. In contrast, knockout of Chst14/D4st1, responsible for adding 4-sulfate to dermatan, results in impaired spatial learning and memory [52].

1.3.3.5. Higher sophisticated neurological functions: neuroplasticity and motor functions. While the fundamental biological roles of nerural specific GAGs and PGs have been discussed, this section explores the deeper contributions to higher neurological functions in the CNS following injuries and in disease states. Neural specific PGs further support sophisticated higher neurological functions and either prohibit or guide regeneration of those higher functions and destruction or reestablishment of synaptic connections following central nervous system injury and within neural degenerative disorders. Neural specific PGs are not merely structural components of the ECM but rather play an imperative role in higher complex neurological processes. Composition of GAGs and PGs in the neuronal ECM and within PNNs change immensely during development, post trauma, and throughout life through normal ageing processes. These changes in PG compositions can lead to alterations in PNN regeneration, plasticity, and the CNS's ability to function at higher processing levels and can result in disease states, loss of neural plasticity, memory, and eventual loss of gross motor functions in extreme cases [34]. Previous studies provide evidence that in damaged or disease states, an increase in expressed MMPs leads to damage of PGs and disruption of the PNNs, which can expose neurons within the PNNs to higher oxidative stresses and a reduction of neurons and synaptic activity in that region [59]. Additionally, PGs modulate neural plasticity of synaptic connections and the formation of PNNs in development and following trauma, which are fundamental to memory, learning, and motor functions [60]. The exact mechanisms and interplay between different neural PGs in higher sophisticated functions remains unknown, but many connections between neural ECM composition and structure and higher neurological functions have been established.

Different PGs and GAGs show varying roles in the control of higher sophisticated neurological functions. For example, HA shows roles in controlling injury induced plasticity, especially post trauma [61]. CSPGs, as discussed prior, regulate synaptic plasticity underpinning learning and memory. HSPGs, on the other hand, regulate motor functions by modulating the activity of growth factors through sequestration and controlled release of neurotropic factors. HSPGs and CSPGs such as neurocan, perlecan, biglycan, testican, decorin and brevican are upregulated following CNS insult and assist in guiding the reestablishment of neurological connections of different types of neurons. Furthermore, in degenerative disease states and neuroligcal disorders including Alzheimer's disease, Parkinson's disease (PD), schizophrenia, and dementia, neural specific PGs' composition changes to include neurocan, brevican, syndecans, glypicans, neuroglycan, perlecan, appican, NG2, and decorin. Understanding the interplay of the neuronal ECM and higher sophisticated neurological functions is crucial to developing targeted material therapies to help guide neural regeneration post traumatic brain injury and with individuals experiencing neurodegenerative disorders. Table 1 below lists neural specific proteoglycans along with possible biological and higher sophisticated neurological functions. Further connections are discussed between neural GAGs, PGs, and CNS injury and disease states in following sections followed by applying that knowledge towards designing GAG hydrogel-based materials for guided CNS therapy, *in vitro* modelling, and in vivo CNS repair applications (see Table 2).

Table 1
Summary of proteoglycans function in CNS.

Proteoglycans	Determined GAGs	Location	Function	Reference
Versican	CSPG DSPG	ECM	- Inhibition of neurite outgrowth and axonal regeneration - Inhibition of axonal growth in the adult CNS (V2) - promotion of neurogenesis and induction of neural differentiation (V1) - Regulation of neurite outgrowth and synaptic transmission of hippocampal neurons - Suppression of axonal plasticity - Memory retrieve - Expression of versican in brain tumors, such as gliomas, medulloblastomas, schwannomas, neurofibromas, and meningiomas	[24,27]
Neurocan	CSPG	ECM	- Formation of PNNs in the auditory brainstem during postnatal development - Regulates regeneration of damaged tissue - Inhibition of neurite outgrowth and axonal regeneration in the glial scar - Regulation of neural migration and axonal development in the cerebral cortex - Mental disorders, such as schizophrenia	[27,29]
Brevican	CSPG	ECM (GPI anchored)	- Neural regeneration after CNS injury - Promotion of protumor effects in glioma - Alzheimer's disease and expression at different forms of dementias - Synaptogenesis during postnatal development - Memory retrieve - Regulation of axon growth and neural cell behavior	[27,29]
Aggrecan	CSPG KSPG	ECM	- Regulation of neural crest cell migration during embryonic development - Regulation of astrocyte differentiation and control of glial cell maturation during brain development - Neuroprotective roles in PNN - Regulation of neuronal differentiation and synaptic plasticity - Synapse formation - Promotion of axonal growth - Axonal regeneration and functional recovery after spinal cord - Memory - Cognitive learning	[24, 62–64]
Syndecans	HSPG CSPG	Cell Surface (Transmembrane)	- Neurodegeneration, Alzheimer's disease - Axonal growth and brain development - Proliferation of neural progenitor cells during cortical development - Axon guidance and synapse development - Neuronal migration	[20, 65–67], [68,69]
Glypicans	HSPG CSPG	Cell Surface (GPI Anchored)	- Axonal regeneration - Neurodevelopmental and psychiatric disorders, such as ASD neuroticism, and schizophrenia - Synapse formation and development - Neurogenesis - Signaling pathways regulators and synaptic organizers	[37, 70–72]
Phosphacan (PTPζ/ RPTPβ)	CSPG KSPG	Cell Surface (Transmembrane)	- Modulation of neurite outgrowth - Development of spinal cord - Modulation of cellular interactions via heterophilic mechanisms - Self-renewal and maintenance of the neural stem cell niche - Evolving astroglial scar and axonal regenerative failures after CNS, spinal cord and optic nerve and retina - Synaptogenesis - Perineuronal net formation and structure - Establishment and maintenance of retinal lamination - Mossy fiber outgrowth and regeneration in rat hippocampal slice cultures	[30, 73–77]
Neuroglycan-C	CSPG	ECM	- Synaptic plasticity - Schizophrenia - Neurites Formation - Telencephalic Functions - Retinal Neural Network Formation	[38,78], [79,80]
Perlecan	HSPG	Pericellular/Basement Membranes	- Neural repair in tissues and blood–brain barrier following ischemic stroke - Promoting neural stem cell self-renewal and neurogenesis - Developmental neurogenesis - Alzheimer's disease and amyloid disorders - Neural Stem/Progenitor Cell proliferation, neurite extension, and regenerative process in CNS injury	[81,82], [83,84], [85,86]
Biglycan	DSPG CSPG	EMC	- Immunoreactivity in the nuclei of spinal cord and cerebrum sections - Inhibition of neurite outgrowth of sensory neurons - Enhancement of survival of neocortical neurons - Neuroinflammatory responses by promoting M1 microglial activation in - Role in early brain injury after subarachnoid hemorrhage - Memory-promoting effects - Improvement of age-related learning deficits	[87,88], [89,90]
Appican	CSPG	ECM	- Neurite outgrowth or regeneration in Alzheimer disease	[43]
Testican	HSPG CSPG	ECM	- Promotion of axons regeneration in reactive astrocytes after injury - Regulation of CNS development - Formation and maintenance of neuronal structures	[44]

(*continued on next page*)

Table 1 (*continued*)

Proteoglycans	Determined GAGs	Location	Function	Reference
NG2	CSPG4	Cell surface (Transmembrane)	- Regulator of translation in OPCs - Role in microvascular changes in patients with Alzheimer's disease - Synaptic Plasticity - Inhibitor or Stimulator of Axon Growth - Effects on psychiatric and behavioral disorders such as schizophrenia	[32]
Agrin	HSPG	ECM	- Assembly of the postsynaptic structures in the neuromuscular junction - Inhibition of neuronal outgrowth - Establishment of axon pathways	[27,91] [92]
Decorin	CSPG DSPG	ECM	- Promotion of axonal regeneration in sensory neurons - Modulation of neuroinflammation - Axonal regeneration - Role in Alzheimer's disease - Role in fibro-glial scar in Multiple Sclerosis	[45,77] [46]

1.4. GAGs in diseased states and post CNS injury

Traumatic Brain injuries (TBI) including head trauma, stroke, and other major traumas and spinal cord injury (SCI) pose a major health problem to the global population without any recourse. In the US alone, 1.4 million people suffer from some form of TBI every year resulting in 50,000 deaths from head injuries. The initial injuries cause direct neuronal loss, and later complications from the TBI arise during a secondary cascade of events that cause further damage and atrophy in the CNS surrounding the pericontusion region [93]. The underlying pathophysiological events following TBIs and SCIs remain largely unknown, and therapeutic options to treat, heal, and regenerate the CNS post TBI and SCI continue to be severely limited [94]. GAG based hydrogels offer ideal properties to be potential candidates for both *in vitro* research platforms for TBI and CNS models and to be delivered in-vivo as therapeutic matrix to guide regeneration and heal the brain ECM following TBI and CNS injuries. GAGs have grown in popularity for their use as *in vitro* scaffolding materials and building blocks for a variety of tissue types [95,96]. GAGs have also gained traction as materials for *in vitro* neuro-regeneration and degeneration modelling and as in vivo therapeutic materials for traumatic brain injury repair due to their innate properties that mimic and support the brain ECM.

Following a TBI or major CNS damage due to stroke, SCI, concussion, or physical accident and trauma, the composition of the brain ECM and spinal cord changes in response to those injuries [97]. Additionally, the CNS and spinal cord have limited natural healing capabilities and certain CSPGs upregulated post TBI or SCI prevent neuronal regrowth and repair to limit further damage. GAGs further play a special role in trauma response, recovery, and healing. CS and CSPGs specifically have been shown to have altered sulfation patterns following TBI in addition to being upregulated [94,98]. CS and CSPGs sulfation patterns further affect the recovery of the CNS and spinal cord post TBI as the sulfation patterns change the binding affinities towards various growth factors and inflammatory responses molecules. CSPGs present in glial scars or contusions further inhibit axonal sprouting and limit the healing and regeneration process in chronic stages of the injury [13]. Furthermore, GAGs, specifically CS and CSPGs, contribute to brain tissue swelling behavior driving edema following TBI or related symptoms [99].

Due to the limited ability of the native CNS to repair itself following a TBI, GAG based injectable hydrogels offer a possible solution to help guide and support regeneration and healing. By tailoring the hydrogel components, design, and specific GAG and PG ratios, neuronal sprouting, infiltration, and synaptic connectivity can be directed to eliminate glial scarring and provide full CNS function and connectivity. Furthermore, GAGs offer controlled binding sites for local growth factor delivery, inflammatory response control, and the physical cues to aid in neural stem cell infiltration, maturation, and even the possibility for direct neural stem cell delivery to the pericontusion area by injection [100–102].

1.5. Role of GAGs in suppressing neuroinflammation, glial scarring and stimulate wound healing

1.5.1. Role of hyaluronic acid in regulating in glial scarring

Neuroinflammation after brain injury mediated by activated microglia or inflammatory macrophages alters astrocyte function leading to cascade of events initiating astrocyte abandonment of neuronal processes causing secondary injury and glial scarring. Thus, limiting the inflammation can prevent secondary damage of CNS. GAGs in the healthy and diseased brain are the major regulator of homeostasis and upon injury it regulates neuroinflammation, wound healing and glial scarring. The polysaccharide composition of GAGs and the sulfation pattern play diverse role in brain injury and healing, thus understanding the inherent bioactivity of GAGs are of paramount importance for engineering functional scaffolds for disease modeling or delivering therapeutic cells for mitigating neuroinflammation and glial scarring. Among different GAGs, HA is one of the major regulators of inflammation and wound healing. HA expression is upregulated in fetal injury that promote scarless fetal wound healing, by suppressing inflammation and excessive collagen deposition and inhibit platelet degranulation [103]. High molecular weight HA is immunosuppressive and promotes healing of diabetic wound ulcers by upregulating IL-10 produced by adult fibroblasts and support scarless wound healing. Low molecular weight HA (<50 kDa) on the other hand is proinflammatory and pro-angiogenic and recruits immune cells to the wound bed and initiate early wound repair. Upon injury the cells produce factors such as MMPs and radical oxygen and nitrogen species fragment the high molecular weight HA to low molecular weight fragments and trigger inflammation and initiate the healing process. Highly crosslinked HA gels mimic the function of high molecular weight HA and are successfully used for suppressing glial scarring after brain or SCI. High molecular weight HA prevent glial scarring by reducing cell proliferation suppressing the production of CSPGs, thus suppressing astrocytes and immune cell activation [104]. Implantation of 3 % HA gel to a cortical defect created in the brain of Sprague-Dawley rats inhibited glial scarring by reducing the number of glial cells and decreasing the thickness of gliosis [105]. HA gels also display neuroprotective effects in hemisection spinal cord injury by decreasing the magnitude of secondary injury and reduction in disorganized scar tissue formation and the retention of neurons near and above the lesion [106].

Therefore, the implementation of HA gels has the potential to effectively mitigate glial scar formation during surgical interventions in the central nervous system (CNS), which could significantly reduce the occurrence of postoperative or posttraumatic seizures. The application of crosslinked HA gels has been shown to modulate the formation of glial scars by inhibiting the activation of astrocytes and immune cells, consequently preserving nearby neuronal cells in the vicinity of the lesion site. Furthermore, the remarkable healing properties of HA render it an essential constituent in various commercially available wound dressing products.

Table 2
Summary of engineered GAGs based 3D hydrogels for CNS regeneration.

Hydrogel composition	Crosslinking chemistry	Cells and biochemical content	Observations	Injury model	Ref.
HA + SA	- Ionic crosslinking with $CaCO_3$	- hUC-MSCs	- hUC-MSC-loaded hydrogel enhanced functional recovery by promoting cell survival and proliferation. - Hydrogel offered nutrition supply and suppressed immune response. - Hydrogel promoted proliferation and regeneration of endogenous nerve cells	TBI	[140]
PCNs (HS or CS) in HA-matrix	- Schiff base formation with ADH	- Growth factors SDF-1α and bFGF	- Hydrogel reduced the infarct cavity volume by offering structural support. - Hydrogel offered sustained release of SDF-1α and bFGF which promoted endogenous neurogenesis and angiogenesis. - Adding HA-matrix decreased astro-gliosis and promoted immature neuron formation.	Stroke	[141]
HA + heparin + gelatin	- Indirect crosslinking with PEGDA	- NPCs	- No difference in cell migration between encapsulated cells and bare cells was detected. - Hydrogel promoted NPCs survival. - Hydrogel did not affect the differentiation profile of NPCs. - Hydrogel had no clear effect in angiogenesis or neovascularization. - Hydrogel decreased inflammatory reaction.	Stroke	[142]
HA modified with RGD	- Michael type addition	- iPS-NPC	- Adding RGD to the structure promoted cell spreading and migration. - Hydrogel did not increase cell survival, but it did promote NPC differentiation to neuroblasts. - Hydrogel prevented endogenous cell migration to the infarct area.	Stroke	[143]
CS	- Photo-crosslinking of methacrylated CS-A	- NPCs	- Hydrogel improved angiogenesis and vascular density. - Encapsulating NPCs in hydrogel promoted cell survival. - Hydrogel increased the total number of differentiated neurons. - Adding bFGF neutralizing antibody to hydrogels negated the improvement in vascularization and functional recovery. –	Stroke	[144]
HA + poly-L-lysine (PLL)	- Indirect crosslinking where HA was crosslinked with PLL with EDC	- nogo-66 receptor antibody (antiNgR) –	- Both hydrogels (with and without antiNgR) promoted angiogenesis and inhibited the formation of complex scar, but HA-PLL/antiNgR hydrogel potentially diminished the chemical barrier brought by astrocytic reaction processes. - HA-PLL/antiNgR hydrogel supported axonal regeneration compared to bare HA-PLL. –	SCI	[145]
HA-PH modified with RGD	- Enzymatic crosslinking with HRP and hydrogen peroxide (H_2O_2) - Two crosslinking strategies: prior to transplantation and *in situ* –	- hWJ-MSCs	- Adding RGD improved cell adhesion, but it was further improved with adding fibrinogen to achieve satisfactory adhesion properties. - HA-PH-RGD hydrogels bridged the lesion cavity, promoted axonal ingrowth to the lesion and vascularization. - Both crosslinking methods resulted in similar results in vascularization and cellular behavior.	SCI	[146]
CS	- Photo-crosslinking of methacrylated CS-A	- NCSs	- Hydrogels were effective in controlling the differentiation of NSCs which lead to reduced fibroglia formation and promoted neurogenesis.	SCI	[123]

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Table 2 (*continued*)

Hydrogel composition	Crosslinking chemistry	Cells and biochemical content	Observations	Injury model	Ref.
			- Compared to bare NCS transplantation, including CSMA hydrogel into the injured spinal cord reduced allodynia. –		
HA-TG (HA-VS + TG + MMP degradable peptides)	- Enzymatic transglutaminase (TG) and coagulation factor XIII (FXIIIa)	- Neurons extracted from Cortices of E17 Wistar Rat embryos	- Fully injectable and attaches covalently to ECM proteins like fibrinogen and fibrin to directly bind to defects in spinal cord or brain - Grew 3D neuronal cultures with excellent outcomes like strong synaptic connectivity and electrical activity	TBI Neuronal *in vitro*	[147]
HA-Tyramine (HT)	- Dual Enzymatic with Galactose Oxidase (GalOx) and Horseradish peroxidase (HRP)	- Bone Mesenchymal Stem Cells (BMSC) with Nerve growth factors (NGF)	- HT hydrogels with BMSCs and NGF increased neuro function and repair in TBI mice after 28 days as compared to Normal Saline injection group	TBI *in vitro* and in vivo	[148]
HA + Dopamine (DA) + Propionic acid (HPA)	- Enzymatic with hydrogen peroxide and horseradish peroxidase (HRP)	- Human mesenchymal stem cells (hMSCs)	- Highly tunable crosslinking times by varying concentration of HRP from 3 to 5 min. - Cytotoxic effects observed with higher HRP amounts due to residual degradation products - successful culture of Human iPSC derived NSCs	CNS Repair and Regeneration	[149]
HA-Spidroin (recombinant spider silk fibroin)	- Thiol reactive covalent crosslinker, Extralink-1	- Human neural progenitor cell s(hNPCs) and human peripheral blood mononuclear cells (hPBMCs)	- Endotoxins present from recombinant spider silk fibroin significantly triggered immune response *in vitro* while HA hydrogels did not induce host immune response. - Method provides platform to detect immunogenicity of biomaterials early on in design process to improve development and production of useable biomaterials for SCI	SCI repair, immune response *in vitro*	[150]
DuraGen Plus (Bovine Dura Mater Collagen and GAGs)	- Commercially available DuraGen membrane comes preformed	- Mixed Glial cultures from CD1 mice cerebral cortices	- DuraGen membrane model mimics in vivo TBI pathology - This model is optimal for complex evaluations of the neuroinflammatory response following TBI.	TBI *in vitro* model	[151]
HA granules from HyStem	- Thiol reactive crosslinker Extralink-1 PEGDA	- hiPSC line 010S-1 from skin biopsy of 18 year old female patient differentiated to NPCs	- Hydrogels supported long term NPC culture and showed high levels of neurite outgrowth. - Optimal hydrogel system for 3D printing CNS brain models	3D printable CNS model with rapid neuronal network formation	[152]
HA-DA-CS	- Hydrazone Crosslinking between covalently linked Aldehyde group and Hydrazide groups	- Regea08/023 (hESCs) and UTA04511.WTs (hiPSCs)	- Injectable Self-Healing and shear thinning hydrogel with optimal biochemical cues to promote neurite outgrowth. - CS and DA components in hydrogel mimic native brain ECM CSPGs and provide binding sites for neurotrophic factors to support neuronal network formation while also providing necessary cues to promote ECM deposition and remodeling.	*In vitro* Brain mimetic scaffold	[124]
Methacrylated CS-GAGs (monosulfated CS-4,CS-6 and disulfated CS-4,6)	- Photocrosslinking with Irgacure-2959	- NSCs and Rat CNS neurospheres	- CS-GAG based hydrogels enhance trophic factor enrichment and increase NSC self-renewal capacity. - Naturally degradable via enzymatic degradation, bind and hold on to anti-inflammatory factors with high affinity, prolong viability of encapsulated stem cells, and enhance NSC self-renewal cycle.	*In vitro* NSC niche	[153]
Fiber like Heparin-maleimide Cryogel	- Photopolymerization with PEGDA, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator and ATTO 610 maleimide	- PC12 pheochromocytoma cells	- Photopolymerizable GAG based cryogels allowed for loading of large amounts of NGF for delayed release over two weeks promoting neurite outgrowth. - Excellent mechanical properties to maintain shape and structure after injection through needle for direct intracranial or spinal cord delivery.	Injectable local growth factor delivery	[154]

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Table 2 (*continued*)

Hydrogel composition	Crosslinking chemistry	Cells and biochemical content	Observations	Injury model	Ref.
Polydopamine modified Geranium phosphide nanosheets in adhesive HA-DA	- Enzymatic Crosslinking with horseradish peroxidase and hydrogen peroxide	- NSCs *in vitro* and in vivo rat spinal cord injury	- HA-DA with conductive Geranium phosphide sheets accelerated differentiation of NSCs *in vitro.* - Iv vivo implanted hydrogel activated NSC neurogenesis and recovered motor function in rats post SCI. - Hydrogel induced immune regulation and endogenous angiogenesis at SCI site.	SCI in vivo model	[155]
Aldehyde HA (HA-CHO) and poly (amidomine) PAMAM/siRNA	- PAMAM Dynamic crosslinking	- Neural progenitor cells	- Injectable self healing HA based hydrogel eased IVD inflammation and degeneration by delivering siRNA STING knockout in vivo - Revealed potential therapeutic target of STING pathway for reducing inflammation in spinal cord defects.	In vivo disc degeneration model	[156]
Imidazole Phosphazene with Arylsulfatase B (I5-ASRB)	- Physical Gel-Sol transition, temperature responsive	- Menengeal Fibroblasts from P1 rat brains	- Injectable hydrogel promotes endogenous formation of fibronectin rich ECM. - Hydrogel containing ARSB enzyme that degrades CSPGs alleviates fibrosis at injection site. - Improved motor recovery function by creating ECM endogenously that bridges spinal cord tissue defects.	SCI Regeneration in vivo	[157]
Chondroitin Sulfate-Gelatin-Polypyrrole	- Dynamic covalent chemistry with Schiff-Base and Borate-diol ester bonds	- NSCs from hippocampi of E14 mouse embryos	- Conductive, injectable, and self-healing hydrogel promoted neuronal differentiation and axon outgrowth in vivo - Enhanced neurogenesis at site of injection and increased motor function recovery post SCI.	SCI Regeneration in vivo	[158]
Methacrylated CS-GAG and HA-Methacrylate loaded with FGF-2	- Photocrosslinked with photoinitiator irgracure	- Primary Rat NSCs and in vivo studies	- CS-GAG hydrogels selectively bind and store FGF2 compared to non-sulfated HA hydrogels. - CS-GAG hydrogel increased in vivo neuroprotection, survival, and proliferation of NSCs after 4 weeks post TBI - CS-GAGs hydrogel implants promote FGF2 retention and increased encapsulated NSC self-renewal capabilities. - CS-GAG hydrogel reduced inflammatory response significantly further lowering astroglia scarring as compared to TBI control and NSC only groups.	TBI	[159]
eCS matrix functionalized with FGF2 and BDNF	- Photocrosslinked with irgracure	- In vivo head injection post TBI	- eCS matrices have physiologically relevant attributes required to control tissue level repair and functional recovery post TBI. - Sulfated GAG constructs improved neuronal connectivity and electrophysiological response in perilesional space in vivo.	TBI in vivo	[160]
Collagen/heparan Sulfate	- UV photocrosslinking and rapid cooling	- NSCs from E14 brains and in vivo spinal sord assessment in Sprague-Dawley rats	- 3D printable collagen/heparan sulfate scaffold enhanced mechanical properties compared with collagen alone along with axonal regeneration and functional recovery post SCI in rats. - Collagen/Heparin scaffolds retained and released bFGF significantly more than Collagen alone.	SCI in vivo	[161]
MeHA with Collagen I	- Photocrosslinking with Irgacure 2959 and LAP	- Rat PC-12, Schwann Cells, and Dorsal Root Ganglia	- MeHA 3D printable bioink provides platform to include other ECM proteins for competitive neurite outgrowth and reproducible *in vitro* test method for neural models.	*In vitro* neural test bed, 3D printed	[162]
Chitosan, HA derivatives, matrigel	- Thermogelation and covalent crosslinking via Michael addition with HA-VS	- SD rat telencephalon NSCs *in vitro*, Spinal cord implantation in vivo	- 3D printable living neural scaffold mimics native spinal cord on micro and macro scale.	SCI	[163]

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Table 2 (*continued*)

Hydrogel composition	Crosslinking chemistry	Cells and biochemical content	Observations	Injury model	Ref.
			- Stimulated parallel white matter of spinal cord providing optimal neuron regeneration and connection. - Implanted hydrogel rapidly restored locomotor function in rats post SCI.		
Glycidyl Methacrylate- HA	- Photopolymerization	- Human Patient derived Glioblastoma stem cells (TS576) and CW468	- 3D printable biochemically and physiologically relevant Glioblastoma model included stiffness patterning to study behavior of tumor cells *in vitro* - Differences between stiff and soft regions in the matrices permitted different invasion and growth behaviors from encapsulated tumor cells - Incorporated HUVECs into model to include modelling of tumor vascularization and stiff matrices enhanced chemo drug resistance in coculture model. - biorthogonal stiffness patterning allows for more precise control over tumor microenvironment and could pave way for future neural models of injury as well.	*In vitro* 3D printed Glioblastoma model	[164]
MeHA with MWCNTs	- Electrospinning fibers	- L-929 Fibroblasts and Lumbar dorsal root ganglia from E11 chick embryos	- Conductive HA based hydrogels with CNTs enhanced neuronal growth - Electrical stimulation provided by the incorporation of CNTs and applying electrical current into the MeHA hdyrogels significantly enhanced neuron growth compared to HA controls –	*In vitro* Neural model	[165]
PEDOT:CSMA:Tannic Acid with GelMA/ PEGDA	- Photopolymerized with LAP	- Neonatal Sprague-Dawley Rat Telencephalons NSCs	- 3D printable and conductive Hydrogel regulated differentiation of NSCs in neurons. - Provides a useful material platform for delivery of NSCs to injury site to improve connectivity and regeneration potential.	*In vitro* 3D bioprinting of electroconductive neural scaffold	[166]

1.5.2. *Role of CS in brain injury, neuroplasticity, memory, and healing*

CS and CSPGs also play significant roles in wound healing mechanisms especially in adult tissue injuries. While HA predominantly contributes to scarless wound healing in fetal tissues, the healing process of adult wound injuries is primarily governed by CSPGs, which lead to the formation of scar tissue [107]. Following CNS injury, the CSPG expression are upregulated, which act as an inhibitor of neurite outgrowth by attenuating axon elongation to promote astrocytic scarring and to inhibit propagation of inflammation and neurite outgrowth [108]. CSPGs are also potent inhibitor of neuroplasticity and axonal growth after SCI by regulating inflammation at the lesion site. Specifically, CPSGs prevents the resolution of inflammation after brain injury by blocking the conversion of pro-inflammatory immune cells to a pro-repair phenotype in rodent models of SCI [109]. Enzymatic digestion of CSPG glycosaminoglycans by sustained delivery of chondroitinase ABC (chABC) at the injury site enhances immune cell clearance and reduces pro-inflammatory protein and gene expression profiles [73]. Thus, enzymatic degradation of CPSGs improve the regeneration and thereby improving functional recovery of SCI in rodent models [110,111]. Although, chABC treatment has shown promising results in several rodent injury models, several challenges remains for clinical translation of this technology [112]. One such challenge is the poor stability of chABC enzyme in physiological temperature, thus requiring thermoresponsive gels to retain its bioactivity [77].

Although CSPGs are the major component of glial scarring and are considered to be an inhibitory molecule for CNS recovery after injury, they also play a pivotal role in the healing of injured spinal cord and the recovery of motor function, by modulating blood-borne monocytes and resident microglia [113]. The enzymatic degradation of CSPGs produce small molecular weight fragments that display neuroprotective effects by modulating neuronal and microglia behavior [114]. Specifically, the disaccharide units from CSPG induced neurite outgrowth and protected against neuronal toxicity and axonal collapse *in vitro*, while the microglia differentiated to a neuroprotective phenotype. This was further verified in the in vivo model, which showed that systemic or locally administration of CS disaccharide protected neurons in mice subjected to glutamate or aggregated beta-amyloid intoxication [114]. The CS fragments also modulate inflammatory response by suppressing T-cell infiltration and microglia activation and facilitated recovery in immune-induced neuropathologies of the CNS, such as experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune uveitis (EAU) [115].

CS sulfation patterns also influence the healing and axonal growth of injured neurons. Two mono-sulfated CS, namely C4S (Chondroitin-4-sulfate or CS-A) and C6S (Chondroitin-6-sulfate or CS-C) are the two main CSs in the CNS [116]. The C4S negatively modulate neuronal regeneration and axonal growth, while C6S stimulate axonal growth [117]. The C6S play a dominant role in maintaining neuroplasticity and memory in aging brain [14]. With age, the C6S to C4S ratio decline [118] leading to formation of inhibitory PNN matrix, thus causing diminished plasticity and memory [116]. Similar to mono-sulfated C6S, the 4,6 disulfated CS or CS-E also display axonal growth promoting properties [8]. Contactin-1, an anchored cell adhesion molecule of the immunoglobulin superfamily binds to CS-E and induces intracellular downstream signaling and leads to axon growth [119].

Thus, the sulfation pattern of CS has profound influence on

neuroplasticity, memory, and glial wound healing. The CS based hydrogels are very useful for neuronal differentiation and neural stem cell (NSC) transplantation. NSC transplantation at the brain lesions exert therapeutic and regenerative effects on SCI, TBI and neurodegenerative diseases [120–122]. However, NSCs preferentially differentiate into astrocytes, with relatively few neurons, thus limiting their therapeutic potential. NSCs cultured within methacrylated CS hydrogel display preferential neural differentiation [123]. Implantation of iPSC derived neuronal progenitors in HA-CS composite gels functionalized with dopamine molecules displayed efficient differentiation of these cells to neurons. Such effect was absent in HA gels without CS, thus demonstrating the importance of CS for culturing human neuronal cells [124].

1.5.3. Role of heparin (HP) and heparan sulfate (HS) in neuroprotection, restoration of BBB leakage, and suppression of brain edema

Heparin and heparan sulfate are other key GAGs that display an inhibitory role to CSPGs.

Low molecular weight heparin, namely heparin hexasaccharide (6-mer) and octasaccharide (8-mer) reduced the extent of glial scar formation in cryo-injured cerebral cortex after single injection of 20 μl (10 mg/mL) in the cerebral cortex [125]. Similarly, Neuroparin, a heparin oligosaccharide C3, effectively cross BBB [126] and displayed a neuroprotective effect against cholinergic lesions induced by intracerebroventricular injection of a specific cholinotoxin, AF64A, in rats [127]. However, high molecular weight heparin did not show such beneficial effects, probably due to limited penetration across BBB [127]. Recently, it was reported that nanoformulation of high molecular weight heparin effectively cross BBB and display glioma targeting ability [128].

Previous studies have shown that heparin infusion has neuroprotective effects in subarachnoid hemorrhage and cerebral ischemia [18]. In humans, slow infusion of low-dose unfractionated heparin to patients with aneurysmal subarachnoid hemorrhage (aSAH) reduced clinical vasospasm and vasospasm-related infarction and improved cognitive outcomes [129,130]. Heparin also display neuroprotective effects by safeguarding the brain endothelial glycocalyx [131] against shedding through the suppression of inflammation, as demonstrated in a model of canine septic shock [132]. The degradation of glycocalyx under different pathological conditions such as septic shock, epilepsy or other neuroinflammatory events cause BBB leakage causing activation of glial cells, brain edema and decline of cognitive functions. Recently, it was demonstrated in murine models of status epilepticus (SE) that heparin reduces the mortality, improves neurological deficits and alleviates brain edema after SE through protection of brain endothelial glycocalyx [133].

The protection of glycocalyx and restoration of BBB after heparin infusion is unrelated to its anticoagulant properties. This ability of heparin is attributed to its inhibitory activity to heparanase, an enzyme responsible for cleaving heparan sulfate and shredding of HSPGs from glycocalyx. Heparanase are overexpressed under several pathological conditions and trigger neuroinflammatory response in ischemic stroke [134] subarachnoid hemorrhage [135]. Heparanase is also upregulated in almost all human cancers including various carcinomas, sarcomas and hematological malignancies [101]. Thus, administration of heparin heals disrupted BBB and suppress neuroinflammation, reduce brain edema, and improve neurologic outcome.

1.6. Role of neurospecific GAGs and PGs in aging and degenerative conditions

Alterations in the composition of neural GAGs and PGs occur naturally as we age over time. At young ages, our central nervous system has high concentrations of non-sulfated GAGs like HA and associated HA binding PGs like aggrecan and versican along with a low abundance of sulfated CSPGs and HSPGs which allows for high levels of neuroplasticity, rapid creation of new neural connections, and higher increases in memory and learning. Neural GAGs and PGs help guide the ability of the central nervous system to heal post trauma, absorb new information, and retain that information during ageing. At the beginning of adulthood, an increase in sulfated PGs, especially CSPGs and HSPGs, leads to neural pruning and maturation of existing connections, leading into the formation of matured adult brain and CNS. Natural ageing of the central nervous system includes increasing sulfation degrees of PGs and GAGs, and aggregations of CSPGs, HSPGs, and further sulfated GAGs at later stages in life can lead to degenerative problems associated with ageing and previous trauma like dementia, Alzheimer's disease, and PD [136,137].

These neural PGs and their GAG counterparts are involved in many biologically important interactions with growth factors, chemokines, morphogens, guidance molecules, survival factors, and other extracellular and cell surface components that play a role in ageing and disease progression at the later stages in life [138]. They further have been linked to the accumulation of alpha-synuclein in PD and Alzheimer's and the increase of amyloid deposits, with neural cell surface HSPGs and CSPGs specifically showing a leading role in the uptake of alpha synuclein aggregates [137,139]. By tailoring the composition of neural specific GAGs and their associated PGs, modeling these complex dynamics of CNS ageing and disease progression *in vitro* could be feasible using tissue engineering strategies.

2. Engineering GAG based 3D hydrogels to mimic and support CNS regeneration

GAG based hydrogels can be used to support CNS regeneration post TBI and for *in vitro* modelling of those ailments. Design parameters that must be considered for crafting tailor GAG based hydrogels to model TBI *in vitro* as well as help support, and initiate CNS regeneration include the following: The hydrogel must have the potential for intracerebral delivery as the bulk of the CNS is within the skull. The hydrogel must provide support structure, incorporate inductive signaling to initiate host cell invasion, and provide cues for neovascularization. Moreover, the hydrogel scaffold should degrade overtime as surrounding native brain tissue heals in the place of the hydrogel. Finally, the hydrogel should guide phenotypic differentiation leading to the dissolution of any glial scarring present, allow for axonogenesis, connectivity, and lastly synapse formation and proper function. The designed hydrogel scaffold should incorporate specific GAG types and possible sulfation patterns to help meet these listed design criteria [167–170].

Researchers around the world have begun investigating GAGs based hydrogel systems as potential candidate materials for CNS injury research and therapies (Table 2). The two main areas of application of these GAG hydrogel systems to both mimic and regenerate the CNS have been in *in vitro* modelling and for injectable therapeutic systems to improve TBI outcomes. For *in vitro* TBI modelling, the main design constraints of the applied GAG biomaterial are to mimic the CNS ECM form and function in both healthy and post TBI disease states to fully recapitulate TBI outcomes. The *in vitro* hydrogels should fully model the in vivo states of the CNS and support neuronal cell growth *in vitro*. Fig. 3 summarizes the applications for GAG based hydrogels for CNS applications. The models can be designed to both mimic the healthy state, controlled development of the TBI disease states, or simply designed to fully recapitulate the contusive damaged state following injury.

For developing injectable GAG based hydrogels for regenerative applications such as spinal cord repair or TBI stabilization and improvement, the design considerations include controlling cell invasion, guiding axonal regeneration, and ultimately supporting and guiding the regeneration of the whole contusion area post TBI using GAGs as the main instructive moieties for the healing and repair process. The hydrogels developed for injection must also have controllable mechanics and be able to be extruded through a needle during injection and have all the while maintaining their mechanical strength to support initial neuronal invasion post injection. The scaffold surface topography and composition must also be devised to help support and stabilize the

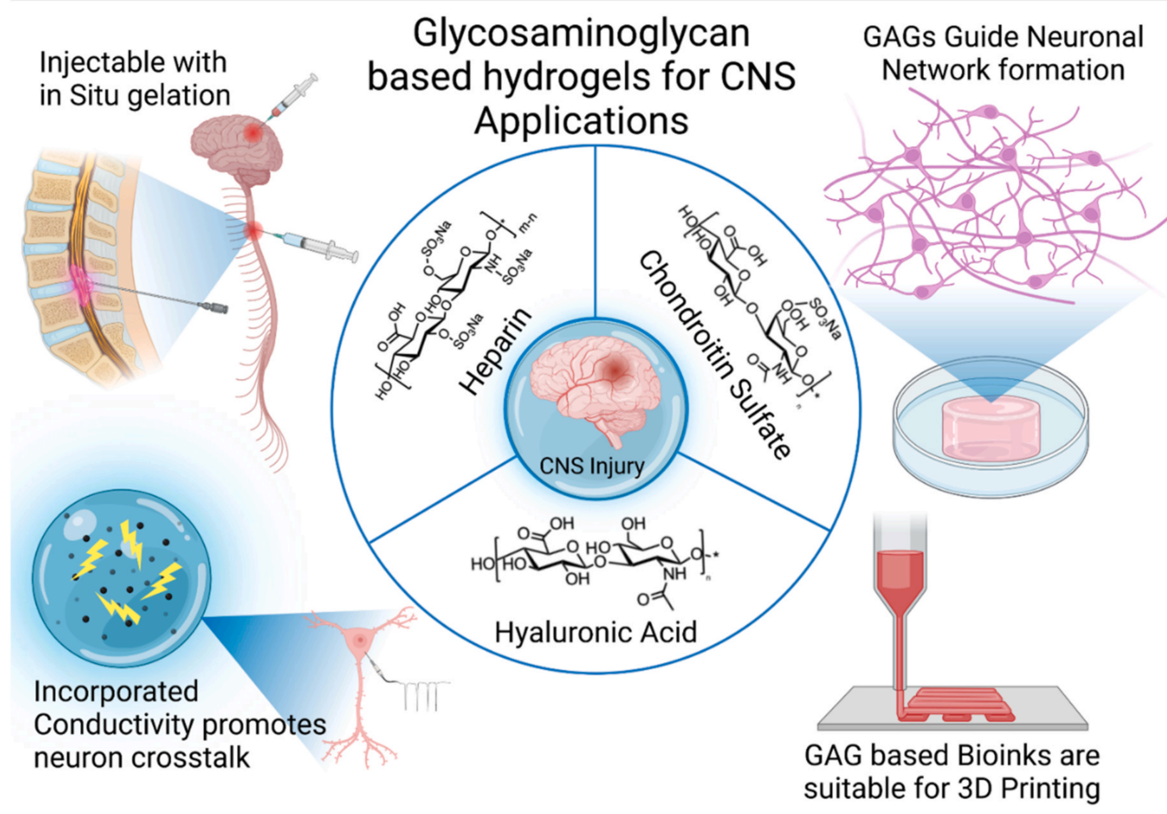

Fig. 3. GAG based hydrogels provide physiologically relevant characteristics for brain modeling and CNS regeneration applications. Injectable, 3D printable, and conductive GAG based hydrogels improve CNS injury outcomes and provide platforms for more accurate *in vitro* modelling of CNS injuries. Created with BioRender.com.

surrounding damaged CNS environment and help spark the healing process [171]. The materials must also follow most of the previously listed design constraints and incorporate instructive cues to guide healing without leading the brain tissue towards glial scarring.

2.1. GAG based 3D scaffolds for in vitro TBI and CNS modelling

GAGs provide excellent biological characteristics to recapitulate the brain physiology in a hydrogel system and can provide biological cues to guide neuronal behavior *in vitro* towards both healthy and disease or damaged states. The 3D nature of GAGs based hydrogels provide intrinsic properties of the brain ECM that more efficiently and accurately model the brain better than previous 2D models and culture methods [172]. Furthermore, by altering the hydrogel composition and possible sulfation patterns on the incorporated GAGs, the designed 3D GAG hydrogel model can be tailored towards the desired CNS disease or healing outcome and can model the effects of damage on neurons in a controlled *in vitro* environment [98,173–177].

The first GAG based models for *in vitro* TBI and CNS modelling are centered around HA based systems as HA provides the backbone structure for the majority of PG complexes in the native brain ECM. Moreover, HA can be easily modified chemically to provide binding sites, attach bioactive molecules, and incorporate chemical or physical crosslinking modalities onto the HA backbone. HA hydrogels, due to the inherent lack of sulfation and cell attachment points, tend to have a neutral effect on cell differentiation and proliferation. In terms of the CNS, HA hydrogels also do not induce local glial scarring, promote angiogenesis, and allow neuronal sprouting and ingrowth into the gel [178–180]. Broguiere et al., in 2016 illustrated successful growth of 3D neuronal networks *in vitro* with an enzymatically crosslinked HA hydrogel system with the inclusion of activated blood coagulation factor XIII (FXIIIa). Their injectable hydrogel provoked unprecedented fast neurite outgrowth, axonal and dendritic guidance, improved synaptic connectivity, and rapid coordinated and long lasting sparking of electrical activity within the 3D model *in vitro* [147]. Furthermore, recent examples of successful HA based model systems include Wang et al., 's 2022 work on HA hydrogels loaded with Bone mesenchymal stem cells (BMSCs) and nerve growth factors (NGF) to treat TBI. Their hydrogel facilitated the survival and proliferation of incorporated neurons and illustrated good biocompatibility [148]. Ngyuen et al. also produced an HA containing hydrogel system for neural regeneration and modeling. Their enzymatically crosslinking hydrogel system further incorporated dopamine (DA) and 3-(4-hydroxyphenyl) propionic acid (HPA) conjugates. They illustrated their hydrogel supports human mesenchymal stem cell and human induced pluripotent stem cell derived neural stem cell proliferation. Further, since DA is known to support the rejuvenation of dopaminergic neurons, the incorporation in to their HA hydrogel shows promise as a model material and possibly as an injectable therapeutic delivery system to treat TBI and CNS damage [149].

HA has also been combined with protein based natural materials as Protein-GAG hybrid hydrogel systems to more closely mimic the brain ECM structure and PG composition. *In vitro* efforts have also led towards the development of models to rapidly screen novel GAG based biomaterials for biocompatibility and their effect on the CNS. Lin et al., in 2021 devised an HA based spider silk fibroin hybrid scaffold to help treat SCIs. They devised *in vitro* experiments to study the short- and long-term immune responses of HA/silk fibroin scaffolds grafted with Human neural stem/progenitor cells in the presence of human immune cells. Their study illustrated that the immune cell activation levels rose in the presence of silk fibroin in the matrix, but not with HA alone. Their study helps pave the way for the creation of models to test the immune response of novel GAG biomaterial constructs quickly *in vitro* [150].

Basit et al., in 2021 further developed a high-throughput *in vitro* biomaterial screening method for penetrating TBI injuries using a facile culture model with all the major CNS cell types and DuraGen Plus membrane derived from bovine dura mater. Their results clearly illuminate the possibility to model the effects of a TBI *in vitro* with all the physiological hallmarks of TBI including immune cell infiltration of the lesion with typical scarring responses. Their model could be further used to rapidly assess novel GAG based biomaterials to study whether they help facilitate healing rather than following the normal fibrosis and scarring pathways post TBI [151].

HA has also been used to begin the development of extrudable hydrogels that have potential both *in vitro* and possibly in vivo in the future. Hsu et al., in 2021's HA based 3D extruded granular hydrogels increased the connectivity and neural network formation of hiPSC

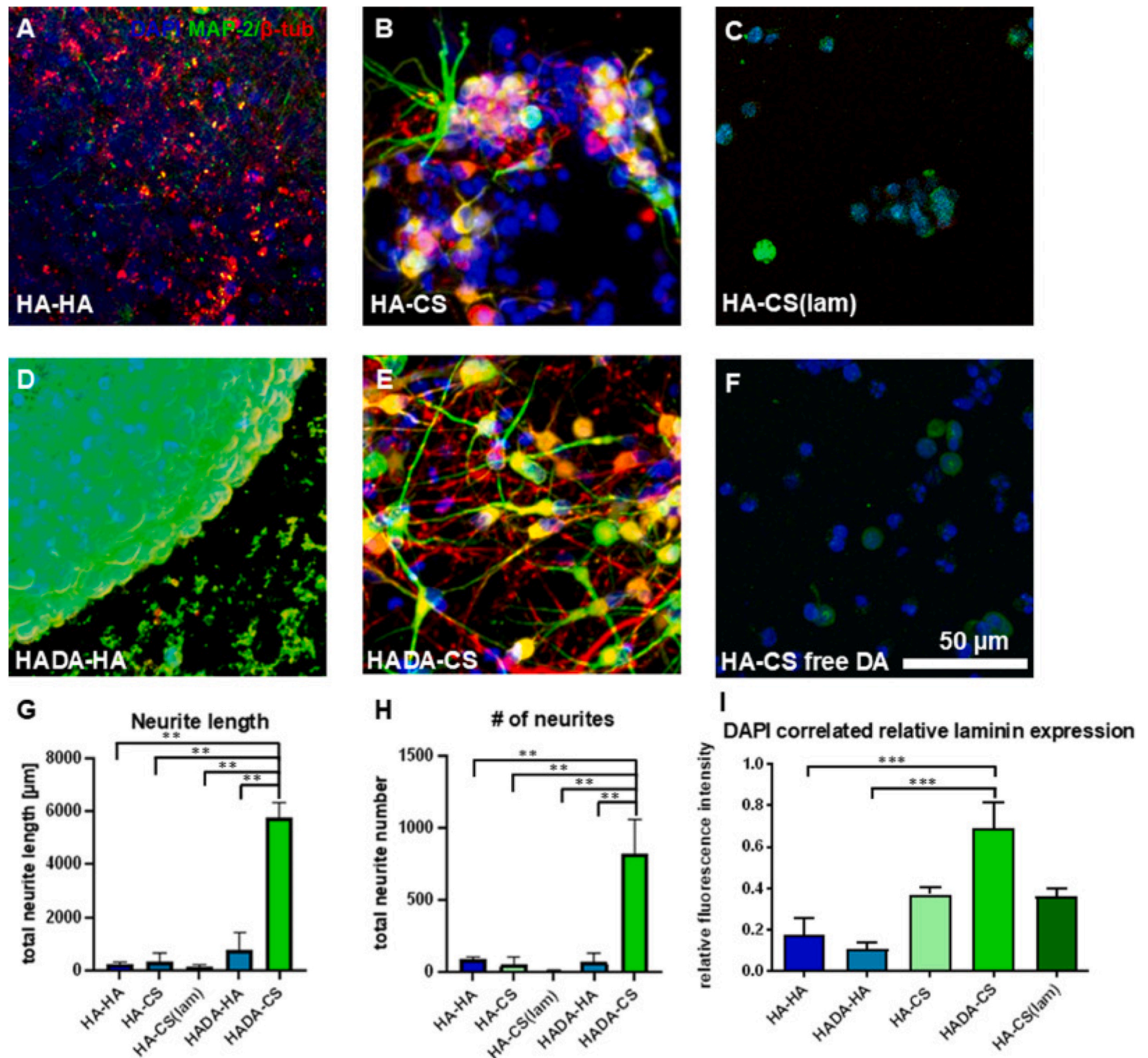

Fig. 4. Neurite outgrowth, network formation, and laminin expression in different gel compositions. hiPSC derived neuronal cells encapsulated within (A) HA-HA (non-viable culture), (B) HA-CS (healthy culture with weak neurite outgrowth), (C) HA-CS(lam) (neurites absent), (D) HADA-HA (strong aggregation), (E) HADA-CS (healthy culture with abundant dendritic presence), (F) HA-CS free DA (neurites absent) hydrogels for 14 days and immunostained against MAP-2 (green, common neuronal marker, staining dendrites) and β-tubulinIII (β-tub, red, a common neuronal marker, staining axons). DAPI (staining cell nuclei) is shown as blue. All images are maximum intensity projections of confocal stacks. (Scalebar = 50 μm) (G) Total neurite length of each neuronal network measured from the 3D render of the confocal stack (N = 5 for each group). (H) The number of neurites (N = 5) of each neuronal network measured from the 3D render of the confocal stack. Five blocks, 150 μm × 150 μm × 150 μm, are analyzed from each gel composition. (I) hESC-derived neuronal cells grown in HA-HA, HADA-HA, HA-CS(lam), HADA-CS, and HA-CS gel were analyzed for laminin protein secretion by correlating laminin fluorescence to DAPI expression in each region of interest (ROI, N = 7 ROIs/sample). Data are expressed as relative laminin staining fluorescence intensity normalized against DAPI intensity. Statistical significance: * = $p < 0.05$, ** = $p < 0.01$ *** = $p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) Figure adapted from Ref. [124] and included under open-access Creative Commons License.

derived neural networks. They depicted a granular extrudable hydrogel system that supported higher neuron counts and longer neurite extensions after 7 days of culture as compared to bulk hydrogels [152]. Further advances with HA based hydrogels have led to the incorporation of other GAGs in addition to HA. Samanta et al., in 2021 showed that injectable HA-CS composite hydrogels dictated 3D neural network formation *in vitro* (Fig. 4). They further employed DA grafting into their HA-CS hydrogels to further support and enhance the biochemical properties of the injectable hydrogel system *in vitro*. Neurons cultured in their hydrogels upregulated Collagen I, Collagen XI and ITGB4 expression important for neuron cell adhesion and ingrowth illustrating a feasible platform for *in vitro* neural network formation, CNS repair, and TBI modeling [124]. The HA-DA-CS group clearly promoted higher connectivity and neural network formation compared to the other groups compiled in their study. The effect of the inclusion of CS and dopamine greatly improved neurite outgrowth as well.

Due to limitations with HA alone as a brain mimetic GAG, CNS and TBI *in vitro* research focus has turned towards using more complex sulfated GAGs and PG complexes, especially CS and CSPG based hydrogels to create TBI and CNS models. HP and HS have also been looked at as possible GAGs for the incorporation into CNS and TBI models. Karumbaiah et al. depicted eloquently how their CS-GAG and CSPG hydrogels create endogenous neural stem cell niches [91]. They investigated CS-GAG hydrogels containing different CS types including monosulfated CS-4, CS-6, and disulfated CS-4,6. They showed that their CS-GAG hydrogels have higher affinity towards trophic factors FGF-2, BDNF, and IL-10 than non-sulfated HA hydrogels. Their results revealed that their CS-GAG based hydrogels can regulate NSC self-renewal and facilitate local growth factor enrichment to maintain the stem cell niche [153]. Jin Zhong et al. described an early iteration of a HA–HS–Collagen hybrid hydrogel that was used to successfully deliver neural progenitor cells to rat brains post stroke, and they showed enhanced survival and diminished cell stress due to the GAG hybrid hydrogel [142].

Further developments to study and control neural stem cell fate and function following stroke *in vitro* were made by Jian et al., in 2018 using hybrid CS and HS hydrogels encapsulated in polyelectrolyte complex nanoparticles. They demonstrated that effective delivery of GAG binding stromal derived factor-1alpha (SDF-1alpha) and basic fibroblast growth factor (bFGF) enhanced the coordination of neurogenesis and angiogenesis post stroke and directed neural stem cell repair and recovery [141]. Newland et al. developed an HP-Polyethylene glycol (PEG) based microscale scaffolds, also termed microcarriers, to recapitulate the neurogenic niche *in vitro*. Their study indicated that the 3D culture environment provided by their hydrogels more closely resembled the in vivo neurogenic niche, and their results exhibited their ability to control and differentiate neural precursor cells (NPCs) better than previously used 2D methods [19]. Newland et al. also investigated injectable GAG based cryogels for local growth factor delivery to support neurogenesis. Their photocrosslinking PEG-maleimide hydrogels formed fiber like heparin based microgels from a sacrificial template. The hydrogels could be loaded with NGF and release the grafted growth factors over a period of 2 weeks, causing neurite outgrowth [154].

2.2. Designing injectable GAGs hydrogels for CNS and TBI injury repair from stroke, physical injury, and spinal cord injury

GAG based biomaterial facilitated CNS regeneration, healing, and repair of the spinal cord and TBI infarctions has grown in popularity in recent years. Many research groups have begun developing injectable GAG based hydrogel scaffolds to direct and coordinate CNS rejuvenation [175,181–183]. GAGs provide instrumental natural cues via sulfation patterns and their biochemical properties that make them ideal candidate materials as a basis for the design of these CNS therapeutic hydrogels. Many research groups have further initiated in vivo models with GAG based hydrogel systems and studies that show clinical potential within the next five to ten years. Following the design considerations from above, the most successful materials illustrate enhanced CNS healing without the presence of scarring following TBI or stroke and they lead to full recovery and connectivity of the SC following SCI. The injected GAG hydrogels quickly stabilize the area of the infarction, initiate healing and regeneration, and limit the native CNS inflammation and normal scarring pathways by supporting the neural stem cell niche with instructive cues [3].

Recent advances in injectable GAG based hydrogels have included in vivo studies showing the recovery of locomotor function, CNS connectivity, immune regulation at the site of injury, and angiogenesis. Xu et al. devised an injectable dopamine grafted hyaluronic acid based two-dimensional-Germanium Phosphide nanosheet reinforced conductive and biodegradable hydrogel that accelerated NSC differentiation *in vitro* and improved and recovered locomotor function once implanted following SCI in vivo in rats. Their hydrogel system promoted immunoregulation, angiogenesis, and neurogenesis in the CNS lesion area where injected by increasing conductivity and providing biochemical structure and support to the cells in the area of infarction [155]. Additional recent GAG based biomaterial advancements include a HA-aldehyde based hydrogel system developed by Chen et al., in 2021 for the purpose of improving siRNA delivery to help reduce intravertebral disc degeneration. By delivering targeted siRNA directly to the site of infarction, they successfully eased intravertebral disc inflammation and slowed degradation of the ECM. Their smart injectable hydrogel system could further improve CNS recovery by incorporating patient specific siRNAs that would initiate and promote CNS regeneration following spinal cord injury [156].

More studies have begun creating more complex and biomimetic GAG based hydrogels that possess multiple functionalities in promoting CNS repair. Park et al. created a CSPG containing dual functional hydrogel system for spinal cord regeneration and sustained release of arylsulfatase B to alleviate fibrosis and support axonal regeneration in rats. They first illustrated the anti-fibrotic effects of their hydrogel system *in vitro* prior to studying the hydrogel injected into rats after spinal cord injury. Their hydrogel successfully reduced fibrotic ECM components present near the site of infarction, improved axonal growth deep into the hydrogel area, and allowed for rejuvenation of locomotor function in rats [157]. Luo et al. combined CS with gelatin in addition to poly-pyrrole as a conductive agent in their hydrogel system to repair traumatic SCIs. Their hydrogels mimic the spinal cord ECM with similar mechanical and conductive properties with injectable sheer thinning and self-healing capabilities. Furthermore, they illuminated *in vitro* that their conductive hydrogels promoted neuronal differentiation, axon outgrowth, and prevented astrocyte differentiation. They further showed full locomotor recovery in Rats following local injection to SCI infarction site, and their hydrogels modulated new axon myelination and growth by activating the PI3K/AKT and MEK/ERK pathways [158].

With respect to TBI treatment, injectable GAG based hydrogel systems have increased in popularity due to the innate favorable biochemical properties that GAGs possess to guide CNS repair and organization. As previously described above, Wang et al. developed an injectable HA based hydrogel system loaded with BMSCs and NGF that proved functional for TBI repair in a mouse model. In addition to their *in vitro* experiments, they further injected their hydrogel into mice following controlled TBI. Their hydrogel exhibited ideal results at the infarction site including improved neurological function recovery, accelerated CNS repair, and neuroinflammation control [148]. Betancur et al. illuminated that methacrylated CS-GAG matrices loaded with FGF2 improve brain tissue repair post TBI. They injected CS-GAG matrices alone and containing NSCs and showed that CS-GAGs alone and CS-GAG-NSC containing hydrogels significantly improved brain tissue repair, prevented NSC differentiation and glial scarring in mice by reducing the local inflammatory response near the site of contusion [159]. Latchoumane et al., in 2021 demonstrated full neuronic circuit repair post TBI using engineered glycomaterial hydrogel implants that

contained methacrylated CS type A-GAGs with neurotrophic factor functionalization as the main proponent. They reported that their CS-GAG based hydrogels significantly augmented cellular repair, gross motor function, and recovery of the rats "grasp to reach" functions. Their CS-GAG hydrogels improved volumetric vascularization, activity regulated cytoskeleton (Arc) protein expression, and sensorimotor connectivity in the pericontusion space. Their results provided clear evidence that GAG based hydrogels can support complex cellular, vascular, and neuronal network formation and repair following TBIs and SCIs [160]. Fig. 5 shows the improvement between the control mice, TBI mice, and eCS implanted mice after 20 weeks post injury.

2.3. GAG-based bioinks for 3D bioprinting for studying brain trauma and diseases

GAG-based hydrogels further provide ideal properties for the development of bioinks for 3D printing for CNS applications. Researchers have started applying more advanced technologies towards neural tissue engineering in the pursuit to more precisely control *in vitro* culture conditions that more relevantly mimic CNS formation, function,

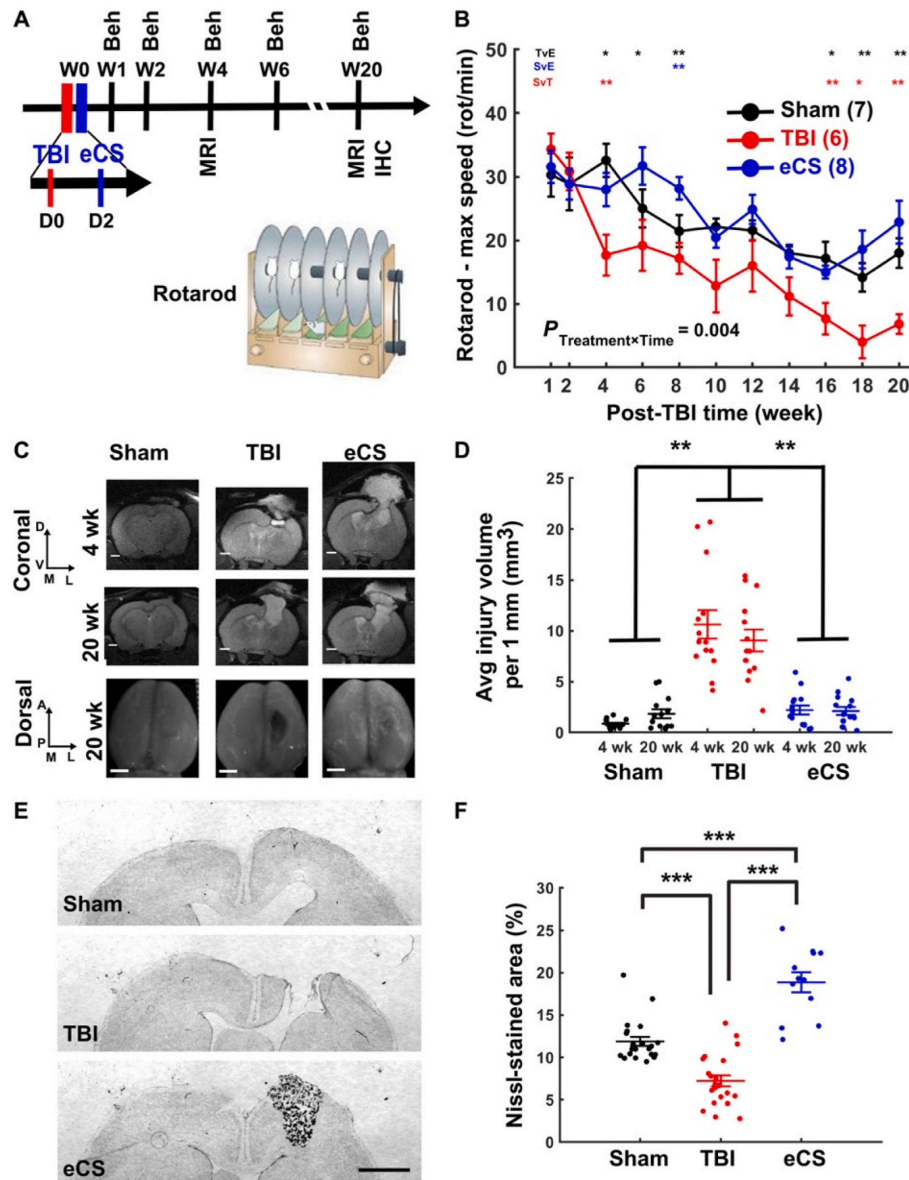

Fig. 5. (A) Experimental schedule. All rats received an sTBI at week 0 (D0). eCS implants were intracortically administered 48 h after sTBI (D2). Following 1 week of recovery, all rats underwent behavioral testing at weeks 1 and 2, and every other week thereafter (4, 6, 8, 10, 12, 14, 16, 18, and 20). (B) The rotarod test was used as a measure of balance and motor coordination. Two-way repeated-measures analysis of variance (ANOVA): $P_{Treatment} = 0.153$; $P_{Time} < 0.001$; $P_{TreatmentxTime} = 0.004$. Post hoc multiple comparisons using Dunn-Sidak correction are shown above each time point for Sham versus TBI (red), Sham versus eCS (blue), and TBI versus eCS (black). (C) Representative T_2-weighted (T2W) MRI images (top) for each treatment group Sham, TBI, and eCS (coronal section); scale bars, 500 μm; top view of the extracted brain (bottom); scale bars, 1 mm. D, dorsal; V, ventral; M, middle; L, lateral; A, anterior; P, posterior. (D) Average injury volume was quantified for each 1-mm slice around the injury and based on the T2W MRI images (mm^3). Two-way repeated-measures ANOVA; treatment factor: $P < 0.001$; time factor: $P = 0.53$; treatment × time: $P = 0.0279$. (E) Representative Nissl-stained coronal sections of rat brain for the Sham, TBI, and eCS groups. Scale bar, 1 mm. (F) Lesion volume quantification using Nissl stain for Sham (six rats, four images per rat), TBI (six rats, four images per rat), and eCS (three rats, four images per rat) groups. One-way ANOVA; treatment: $P < 0.001$. Post hoc least significant difference (LSD), *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Graphs show means ± SEM. Created by Ref. [160] and included under Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) license. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and healing. 3D bioprinting technology allows scientists to create bespoke scaffolds with enhanced functionalities to improve both *in vitro* modelling and drug screening, and ultimately advance in vivo therapeutic outcomes. Types of 3D bioprinting modalities used in CNS tissue engineering include extrusion based, inkjet based, laser assisted, and stereolithography based bioprinting [184].

In addition to the previously described design constraints for GAG-based hydrogels for CNS applications, added design considerations for the creation of a GAG-based bioinks for 3D bioprinting include optimizing the flow behavior and rheological properties to allow the formation of 3D self-supporting constructs while tolerating extrusion through a needle or small nozzle. Bioinks for most 3D bioprinting modalities require shear thinning properties and thixotropic characteristics to allow for easy extrusion and then regaining internal strength to be able to hold its 3D conformation following extrusion. The sheer-thinning behavior, known as thixotropy, has a time-dependent factor where a bioink exhibits low viscosity upon applied sheer during the extrusion process through a nozzle. Thixotropic bioinks decrease their viscosity while undergoing sheer stresses and then later regain internal stability post printing [184,185].

Further mechanical considerations apply to the viscoelastic behavior of the designed GAG-based bioink. Viscoelastic materials follow non-Newtonian mechanics and have both solid like and liquid like states that dictate their internal mechanics. The storage G' and loss moduli G'' are used to describe viscoelastic behavior of a bioink and are obtained via rheometric analysis. The storage modulus G' describes the solid portion or elasticity of the bioink and relates to its shape retention ability and stored elastic energy, whereas the loss modulus G'' illuminates the liquid like viscous flow proportion and energy loss of the material. The quantification of the storage G' and loss G" moduli of a bioink exemplifies a critical step in bioink design. Lastly, viscosity of the bioink must be tailored towards the desired application, output, and bioprinting modality. Low viscosity bioinks increase cell viability but may lose shape retention and definition post printing, while high viscosity bioinks have better shape fidelity but often lead to cell damage and cell death [184,185].

A multitude of research groups have developed 3D printable GAG based bioinks for CNS repair and *in vitro* modelling of CNS injuries and diseases. Chen et al. created a Collagen/Heparin Sulfate composite 3D printable scaffold to repair SCIs in 2017. By combining collagen and heparin sulfate into a composite hydrogel, they established a bioink with superior mechanical properties compared to collagen alone that more closely mimics the in vivo spinal cord microenvironment. They further ameliorated bFGF immobilization and absorption by modifying the heparin sulfate and quantified this effect by studying the release kinetics of bFGF *in vitro*. After 2 months post SCI and hydrogel implantation into the spinal cord lesion site in rats, their collagen/heparin sulfate bioink groups led to significant recovery of locomotor function, an increased amount of neurofilament positive cells compared to collagen alone according to electrophysiological measurements. Their results illuminate the potential for their bioink to regenerate neurological function post SCI in vivo [161]. In 2020, Ngo et al. manufactured a Methacrylated HA (MeHA) based 3D printable bioink for *in vitro* modelling of neural repair processes and competitive biochemical cues. Their work combined 10 mg/mL MeHA with 3 mg/mL collagen-I to form a suitable bioink for neural cells. They demonstrated that their bioink improved the migration, proliferation, and adhesion of Schwann cells encapsulated with beta I immunostaining compared to collagen alone. Further, they created a two-chamber *in vitro* test bed and evaluated neurite extension of dorsal ganglia in response to NGF and GDNF exposure. Their results display promise for their GAG based bioink for use in CNS modelling platforms and in vivo CNS regeneration applications [162]. In 2021, Liu et al. fashioned a 3D printable bioink consisting of Chitosan, HA derivatives, and Matrigel for SCI repair. Their results depict NSC laden 3D printed constructs that mimic the innate SC microenvironment, improve neuronal differentiation, and neural network formation leading to the stimulation of axon regeneration and decreased glial scarring. The in vivo results also indicated significant locomotor function recovery in the SCI model rats after bioink printing into lesion site and demonstrates a capacity for CNS regeneration models and therapeutic potential in the future [163]. Fig. 6 illustrates the 3D printable solution developed by Liu et al. in their study.

More complex *in vitro* models have utilized 3D bioprinting technology to add heterogeneity like varied stiffness, gradients of materials, and cell densities to more closely replicate the in vivo brain physiology. In 2021, Min Tang et al. developed a species matched HA based bioink to model patient derived glioblastoma multiform using a digital light processing (DLP) based bioprinting method. They printed patient specific *in vitro* scaffolds with three distinct material stiffnesses within one construct by modulating the material concentration and printing parameters showing regionally varied biophysical properties like native brain tumor morphology. They illustrated differences in cell phenotype and differentiation profiles between the stiff and soft areas within the scaffold illuminating those cells growing on the stiff areas gravitated towards a mesenchymal phenotype while cells on the soft substrate proliferated and maintained their classical neural phenotype. They also reported that patients with stiffer tumors generally had poorer clinical outcomes, and thus their HA-based bioink scaffolds allowed them to decipher and recapitulate those ECM differences *in vitro*. They further used their 3D bioprinting platform to model patient specific glioblastoma multiform in co-cultures and non-cocultures to examine tumorigenesis *in vitro* including invasion behaviors, changes in cell morphologies, gene expression, angiogenesis, and lastly drug response screening. Their GAG-based bioink system allowed for rapid patient specific modelling of glioblastoma multiform to hunt for possible drug combinations or therapeutic methods that would be effective for specific patients [164]. Their bioink method provides a platform for developing rapid TBI and SCI models as well *in vitro*.

3D bioprinting allows for the creation of more advanced *in vitro* brain models that can help accelerate the development of novel therapeutics for TBI, SCI, and brain diseases. With material modifications or composite mixing, natural GAG-based hydrogel materials provide excellent characteristics for 3D bioprinting and for more accurately recapitulating the brain ECM *in vitro* of TBIs, SCIs, and neural damage resulting from disease.

2.4. GAG-based conductive scaffolds for studying neuronal activity

Another recent trend in brain injury modelling and the development of brain therapeutic materials include the incorporation of conductive materials to improve neuronal connectivity to accelerate and promote nerve regeneration post trauma. Conductive materials embedded within a hydrogel material provide electrical cues to enhance neural cell communication and signaling. In CNS repair, neural cells differentiate to form dendrites and axons while Schwann cells provide the protective insulative material, the myelin sheath. With incorporated conductivity, neural cells can more quickly communicate to one another to form electrical connections between cells, thus accelerating regeneration of the nerve [186]. Conductive materials used in hydrogel systems previously include carbon-based nanoparticles, gold nanoparticles, and conductive polymers. Combining this conductive scaffold approach in coordination with the use of GAG-based hydrogel systems shows promise for future progress of both *in vitro* TBI models and for therapeutic materials targeted towards brain injury, nerve damage, and disease.

Elisabeth Steel et al., in 2020 crafted an HA based nano-filament based electrospun scaffold doped with carbon nanotubes (CNTs) to improve neurite outgrowth and neuronal cell proliferation. By incorporating a very small amount of CNTs, they significantly increased the electrical conductivity of the HA scaffold compared to HA alone. Their results with neuronal cell studies further indicated a significant increase in axon length and sprouting compared to HA scaffolds alone, suggesting

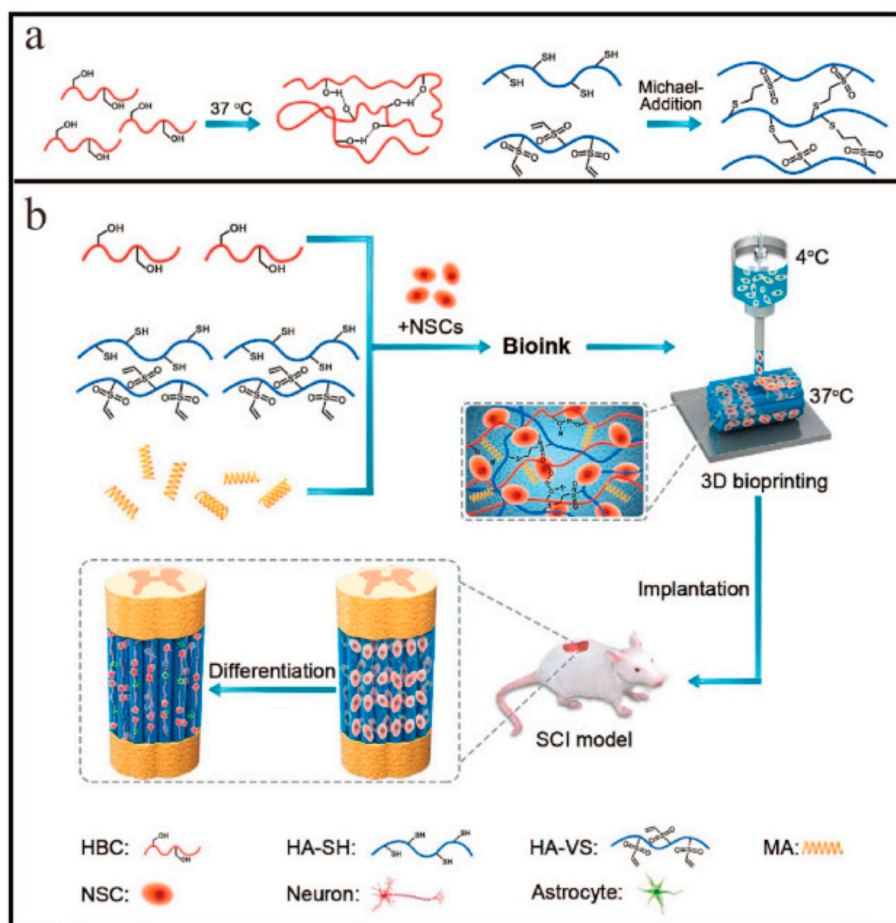

Fig. 6. Schematic representation of the NSC-laden bioprinted neural tissue constructs for in vivo SCI repair. (a) The cross-linking reactions during and after the 3D printing of the HBC/HA/MA bioink and (b) 3D bioprinting of the NSC-laden white matter of spinal cord-like scaffold and its application for in vivo SCI repair. Included with permission via CCC Rights link license #5692980693144 [163].

that the increase in electrical conductivity in the hydrogel enhanced axon growth and neuronal cell proliferation [165]. The previously described HA hydrogel system with two-dimensional germanium phosphide sheets also incorporate electroconductive properties to improve neuronal regeneration and connection post SCI in rats. Their hydrogel successfully accelerated locomotor functional recovery in rats with injection of their reinforced hydrogel compared to controls [155]. The effects of the geranium phosphide in SCI recovery on locomotor function and BBB score in rats was significantly greater with the inclusion of the electroconductive geranium sheets in their injectable hydrogel system as shown in Fig. 7.

Further advancements have been made by Shaoshuai Song et al. in late 2021 using a GAG-based hydrogel system combining 3D bioprinting and incorporating polyphenol conductive components to improve neuronal differentiation and accelerate healing. They modified poly(3,4-ethylenedioxythiophene) (PEDOT) with chondroitin sulfate and tannic acids to stabilize PEDOT in water and to increase its electrical conductivity. The conjugation of CS and Tannic acids to PEDOT provided a crucial element to their brain mimetic bioink to support neuronal electrical crosstalk and provided a platform to enhance neural cell differentiation. They employed cell viability studies to show the neural cells were proliferating post printing and that their electroactive scaffolds provide an enhanced microenvironment for neural cell differentiation. Their material development process also depicts methods to further improve neural cell control and differentiation for nerve injury repair and for neurodegenerative diseases [166].

Increasing conductivity of GAG-based hydrogels can aid in the regeneration of neural networks and neural connectivity by supplementing and adding to the peripheral signals already present at the edge's infarction site. With the addition of electrical connections within a supportive GAG based hydrogel, neurons can more quickly reconnect and regenerate function in the CNS post TBIs.

3. Future perspectives, what needs to be further investigated

The current outlook for GAG based biomaterials as potential therapeutic agents for neuroregeneration post TBI looks promising. GAG based hydrogel systems offer ideal properties to support CNS regeneration and full recovery post TBI and spinal cord repair. GAG based hydrogels can further be designed to be injectable, self-healing, conductive, and neural supportive to eliminate glial scarring and provide support for full CNS recovery. There continues to be a lack of therapeutic options available to patients who have experience a TBI or SCI, and these GAG based hydrogel systems offer hope for drastically improving the clinical outcomes of TBIs.

More studies need to be conducted to learn more about GAG biosynthesis and sequencing full chain GAGs in vivo in different settings to promote and guide TBI recovery and repair. Further work also needs to be done to learn how we can harness innate cell-based GAG biosynthesis to guide wanted therapeutic outcomes in the brain and spinal cord. There remains a lot of information also about how epigenetic factors influence the biosynthesis pathways of GAGs and how specific GAG sulfation patterns lead to various outcomes and cell responses.

Furthermore, more complex systems need to be generated incorporating not only GAGs but also their PG counterparts to fully control and instruct CNS regeneration appropriately. Previous hydrogel systems lack

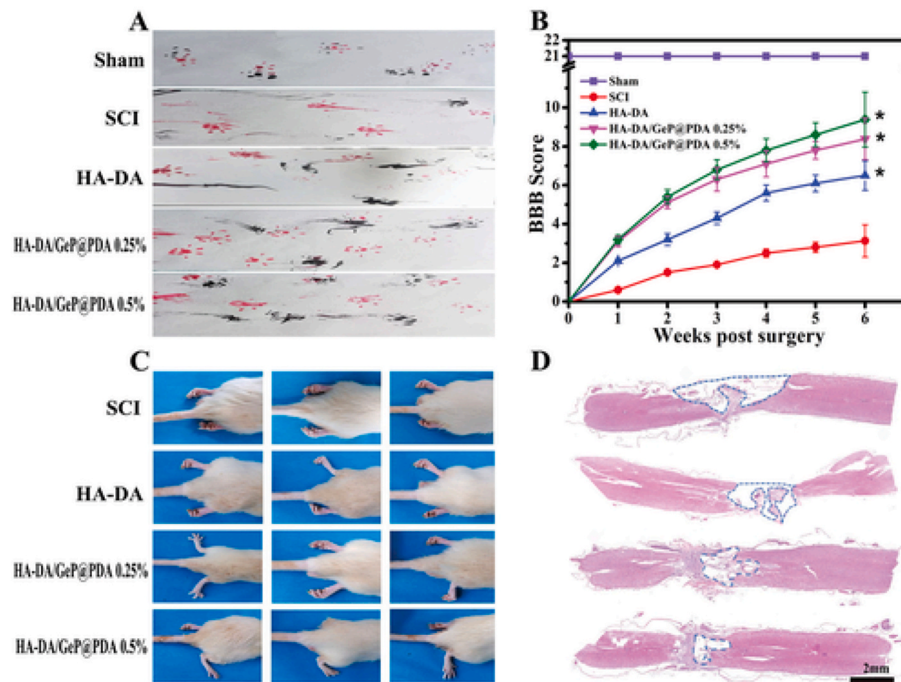

Fig. 7. Motor functional recovery of SCI rats after treatment with the conductive HA-DA/GeP@PDA hydrogels. A) Representative footprints of the rats in the sham, SCI, and hydrogel groups. The fore- and hind-limbs of the animals were inked in red and black, respectively. B) Function recovery of rats on week 6 post-surgery evaluated by BBB scores. C) Typical hindlimbs-spreading status of SCI rats on week 6 post-surgery. D) Histological examination on the longitudinal sections of the spinal cords collected on week 6 post-surgery. *Indicates difference with significance respect to the SCI group ($p < 0.05$). Adapted from Ref. [155] with CCC Rights link permission license #5692980998643. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

complexity, mechanical support, and the biochemical factors to fully provide and promote CNS regeneration, but GAG based hydrogels are now bridging that gap to completely heal TBI and other CNS injuries and guide regeneration of CNS diseased states.

CRediT authorship contribution statement

Austin D. Evans: Writing – review & editing, Writing – original draft. **Negin Pournoori:** Writing – review & editing. **Emmi Saksala:** Writing – original draft, Conceptualization. **Oommen P. Oommen:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

[1] A. Soles, et al., Extracellular matrix regulation in physiology and in brain disease, Int. J. Mol. Sci. 24 (8) (Jan. 2023), https://doi.org/10.3390/ijms24087049. Art. no. 8.
[2] U. Novak, A.H. Kaye, Extracellular matrix and the brain: components and function, J. Clin. Neurosci. 7 (4) (Jul. 2000) 280–290, https://doi.org/10.1054/jocn.1999.0212.
[3] W. Jin, F. Zhang, R.J. Linhardt, Glycosaminoglycans in neurodegenerative diseases, in: G. Lauc, I. Trbojević-Akmačić (Eds.), The Role of Glycosylation in Health and Disease, Advances in Experimental Medicine and Biology. , Cham: Springer International Publishing, 2021, pp. 189–204, https://doi.org/10.1007/978-3-030-70115-4_9.
[4] R. Rauti, N. Renous, B.M. Maoz, Mimicking the brain extracellular matrix *in vitro*: a review of current methodologies and challenges, Isr. J. Chem. 60 (12) (2020) 1141–1151, https://doi.org/10.1002/ijch.201900052.
[5] N.B. Schwartz, M.S. Domowicz, Proteoglycans in brain development and pathogenesis, FEBS (Fed. Eur. Biochem. Soc.) Lett. 592 (23) (2018) 3791–3805, https://doi.org/10.1002/1873-3468.13026.
[6] N. Jovanov Milošević, M. Judaš, E. Aronica, I. Kostovic, Neural ECM in laminar organization and connectivity development in healthy and diseased human brain, Prog. Brain Res. 214 (2014) 159–178, https://doi.org/10.1016/B978-0-444-63486-3.00007-4.
[7] C.P. Mencio, R.K. Hussein, P. Yu, H.M. Geller, The role of chondroitin sulfate proteoglycans in nervous system development, J. Histochem. Cytochem. 69 (1) (Jan. 2021) 61–80, https://doi.org/10.1369/0022155420959147.
[8] S. Miyata, H. Kitagawa, Mechanisms for modulation of neural plasticity and axon regeneration by chondroitin sulphate, J. Biochem. 157 (1) (Jan. 2015) 13–22, https://doi.org/10.1093/jb/mvu067.
[9] S. Miyata, H. Kitagawa, Chondroitin sulfate and neuronal disorders, Front. Biosci. 21 (Jun. 2016) 1330–1340, https://doi.org/10.2741/4460.
[10] P.D. Smith, V.J. Coulson-Thomas, S. Foscarin, J.C.F. Kwok, J.W. Fawcett, GAG-ing with the neuron': the role of glycosaminoglycan patterning in the central nervous system, Exp. Neurol. 274 (Dec. 2015) 100–114, https://doi.org/10.1016/j.expneurol.2015.08.004.
[11] X.M.R. Van Wijk, T.H. Van Kuppevelt, Heparan sulfate in angiogenesis: a target for therapy, Angiogenesis (Oct. 2013), https://doi.org/10.1007/s10456-013-9401-6.
[12] A. Basu, N.G. Patel, E.D. Nicholson, R.J. Weiss, Spatiotemporal diversity and regulation of glycosaminoglycans in cell homeostasis and human disease, Am. J. Physiol. Cell Physiol. 322 (5) (May 2022) C849–C864, https://doi.org/10.1152/ajpcell.00085.2022.
[13] D. Soares da Costa, R.L. Reis, I. Pashkuleva, Sulfation of glycosaminoglycans and its implications in human health and disorders, Annu. Rev. Biomed. Eng. 19 (1) (2017) 1–26, https://doi.org/10.1146/annurev-bioeng-071516-044610.
[14] S. Yang, et al., Chondroitin 6-sulphate is required for neuroplasticity and memory in ageing, Mol. Psychiatr. 26 (10) (Oct. 2021), https://doi.org/10.1038/s41380-021-01208-9, 10.

[15] S. Lemarchant, S. Wojciechowski, J. Koistinaho, Perineuronal nets in neurodegeneration, Oncotarget 7 (48) (Nov. 2016) 78224–78225, https://doi.org/10.18632/oncotarget.13420.
[16] R.V. Iozzo, L. Schaefer, Proteoglycan form and function: a comprehensive nomenclature of proteoglycans, Matrix Biol. 42 (Mar. 2015) 11–55, https://doi.org/10.1016/j.matbio.2015.02.003.
[17] D.M. Whalen, T. Malinauskas, R.J.C. Gilbert, C. Siebold, Structural insights into proteoglycan-shaped Hedgehog signaling, Proc. Natl. Acad. Sci. U. S. A. 110 (41) (Oct. 2013) 16420–16425, https://doi.org/10.1073/pnas.1310097110.
[18] J.M. Simard, et al., Heparin reduces neuroinflammation and transsynaptic neuronal apoptosis in a model of subarachnoid hemorrhage, Transl. Stroke Res 3 (S1) (Jul. 2012) 155–165, https://doi.org/10.1007/s12975-012-0166-9.
[19] B. Newland, et al., Macroporous heparin-based microcarriers allow long-term 3D culture and differentiation of neural precursor cells, Biomaterials 230 (Feb. 2020) 119540, https://doi.org/10.1016/j.biomaterials.2019.119540.
[20] X. Zhou, et al., The HSPG syndecan is a core organizer of cholinergic synapses, JCB (J. Cell Biol.) 220 (9) (Jul. 2021) e202011144, https://doi.org/10.1083/jcb.202011144.
[21] A. Purushothaman, K. Sugahara, A. Faissner, Chondroitin sulfate 'wobble motifs' modulate maintenance and differentiation of neural stem cells and their progeny*, J. Biol. Chem. 287 (5) (Jan. 2012) 2935–2942, https://doi.org/10.1074/jbc.R111.298430.
[22] A. Peters, L.S. Sherman, Diverse roles for hyaluronan and hyaluronan receptors in the developing and adult nervous system, Int. J. Mol. Sci. 21 (17) (Jan. 2020), https://doi.org/10.3390/ijms21175988. . 17.
[23] N. Siddiqui, K. Oshima, J.A. Hippensteel, Proteoglycans and glycosaminoglycans in central nervous system injury, Am. J. Physiol. Cell Physiol. 323 (1) (Jul. 2022) C46–C55, https://doi.org/10.1152/ajpcell.00053.2022.
[24] A.J. Hayes, J. Melrose, Neural tissue homeostasis and repair is regulated via CS and DS proteoglycan motifs, Front. Cell Dev. Biol. 9 (Aug. 2021) 696640, https://doi.org/10.3389/fcell.2021.696640.
[25] T.N. Wight, et al., Versican—a critical extracellular matrix regulator of immunity and inflammation, Front. Immunol. 11 (Mar. 2020) 512, https://doi.org/10.3389/fimmu.2020.00512.
[26] S. Bierbaum, V. Hintze, D. Scharnweber, 2.8 Artificial extracellular matrices to functionalize biomaterial surfaces, in: Comprehensive Biomaterials II, Elsevier, 2017, pp. 147–178, https://doi.org/10.1016/B978-0-12-803581-8.10206-1.
[27] Z. Shabani, et al., Modulatory properties of extracellular matrix glycosaminoglycans and proteoglycans on neural stem cells behavior: highlights on regenerative potential and bioactivity, Int. J. Biol. Macromol. 171 (Feb. 2021) 366–381, https://doi.org/10.1016/j.ijbiomac.2021.01.006.
[28] S. Schmidt, T. Arendt, M. Morawski, M. Sonntag, Neurocan contributes to perineuronal net development, Neuroscience 442 (Aug. 2020) 69–86, https://doi.org/10.1016/j.neuroscience.2020.06.040.
[29] T. Fontanil, Y. Mohamedi, J. Espina-Casado, Á.J. Obaya, T. Cobo, S. Cal, Hyalectanase activities by the ADAMTS metalloproteases, IJMS 22 (6) (Mar. 2021) 2988, https://doi.org/10.3390/ijms22062988.
[30] J. Garwood, N. Heck, F. Reichardt, A. Faissner, Phosphacan short isoform, a novel non-proteoglycan variant of phosphacan/receptor protein tyrosine phosphatase-β, interacts with neuronal receptors and promotes neurite outgrowth, J. Biol. Chem. 278 (26) (Jun. 2003) 24164–24173, https://doi.org/10.1074/jbc.M211721200.
[31] A.J. Hayes, B.L. Farrugia, I.J. Biose, G.J. Bix, J. Melrose, Perlecan, A multi-functional, cell-instructive, matrix-stabilizing proteoglycan with roles in tissue development has relevance to connective tissue repair and regeneration, Front. Cell Dev. Biol. 10 (Apr. 2022) 856261, https://doi.org/10.3389/fcell.2022.856261.
[32] Y.A. Chelyshev, I.M. Kabdesh, Y.O. Mukhamedshina, Extracellular matrix in neural plasticity and regeneration, Cell. Mol. Neurobiol. 42 (3) (Apr. 2022) 647–664, https://doi.org/10.1007/s10571-020-00986-0.
[33] J. Lin, M. Duan, N. Lin, W. Zhao, The emerging role of the chondroitin sulfate proteoglycan family in neurodegenerative diseases, Rev. Neurosci. 32 (7) (Nov. 2021) 737–750, https://doi.org/10.1515/revneuro-2020-0146.
[34] H. Cui, C. Freeman, G.A. Jacobson, D.H. Small, Proteoglycans in the central nervous system: role in development, neural repair, and Alzheimer's disease, IUBMB Life 65 (2) (Feb. 2013) 108–120, https://doi.org/10.1002/iub.1118.
[35] E. Steiner, et al., The heparan sulfate proteoglycan agrin contributes to barrier properties of mouse brain endothelial cells by stabilizing adherens junctions, Cell Tissue Res. 358 (2) (Nov. 2014) 465–479, https://doi.org/10.1007/s00441-014-1969-7.
[36] X. Xian, S. Gopal, J.R. Couchman, Syndecans as receptors and organizers of the extracellular matrix, Cell Tissue Res. 339 (1) (Jan. 2010) 31–46, https://doi.org/10.1007/s00441-009-0829-3.
[37] K. Kamimura, N. Maeda, Glypicans and heparan sulfate in synaptic development, neural plasticity, and neurological disorders, Front. Neural Circ. 15 (Feb. 2021) 595596, https://doi.org/10.3389/fncir.2021.595596.
[38] A. Ledonne, N.B. Mercuri, On the modulatory roles of neuregulins/ErbB signaling on synaptic plasticity, IJMS 21 (1) (Dec. 2019) 275, https://doi.org/10.3390/ijms21010275.
[39] K. Nakanishi, et al., Identification of neurite outgrowth-promoting domains of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan, and involvement of phosphatidylinositol 3-kinase and protein kinase C signaling pathways in neuritogenesis, J. Biol. Chem. 281 (34) (Aug. 2006) 24970–24978, https://doi.org/10.1074/jbc.M601498200.
[40] Z. Ying, et al., Biglycan gene connects metabolic dysfunction with brain disorder, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1864 (12) (Dec. 2018) 3679–3687, https://doi.org/10.1016/j.bbadis.2018.10.002.
[41] K. Tsuchida, et al., Appican, the proteoglycan form of the amyloid precursor protein, contains chondroitin sulfate E in the repeating disaccharide region and 4-O-sulfated galactose in the linkage region, J. Biol. Chem. 276 (40) (Oct. 2001) 37155–37160, https://doi.org/10.1074/jbc.M105818200.
[42] M.N. Pangalos, S. Efthimiopoulos, J. Shioi, N.K. Robakis, The chondroitin sulfate attachment site of appican is formed by splicing out exon 15 of the amyloid precursor gene, J. Biol. Chem. 270 (18) (May 1995) 10388–10391, https://doi.org/10.1074/jbc.270.18.10388.
[43] J. Shioi, et al., The Alzheimer amyloid precursor proteoglycan (appican) is present in brain and is produced by astrocytes but not by neurons in primary neural cultures, J. Biol. Chem. 270 (20) (May 1995) 11839–11844, https://doi.org/10.1074/jbc.270.20.11839.
[44] S. Chen, et al., Regulation of SPARC family proteins in disorders of the central nervous system, Brain Res. Bull. 163 (Oct. 2020) 178–189, https://doi.org/10.1016/j.brainresbull.2020.05.005.
[45] A. Arjunan, J. Song, Pharmacological and physiological roles of adipokines and myokines in metabolic-related dementia, Biomed. Pharmacother. 163 (Jul. 2023) 114847, https://doi.org/10.1016/j.biopha.2023.114847.
[46] N. D'Ambrosi, S. Apolloni, Fibrotic scar in neurodegenerative diseases, Front. Immunol. 11 (Aug. 2020) 1394, https://doi.org/10.3389/fimmu.2020.01394.
[47] C. Ohtaka-Maruyama, et al., RP58 regulates the multipolar-bipolar transition of newborn neurons in the developing cerebral cortex, Cell Rep. 3 (2) (Feb. 2013) 458–471, https://doi.org/10.1016/j.celrep.2013.01.012.
[48] X. Tan, S. Shi, Neocortical neurogenesis and neuronal migration, WIREs Developmental Biology 2 (4) (Jul. 2013) 443–459, https://doi.org/10.1002/wdev.88.
[49] N. Maeda, Proteoglycans and neuronal migration in the cerebral cortex during development and disease, Front. Neurosci. 9 (Mar) (2015), https://doi.org/10.3389/fnins.2015.00098.
[50] F.E. Poulain, H.J. Yost, Heparan sulfate proteoglycans: a sugar code for vertebrate development? Development 142 (20) (Oct. 2015) 3456–3467, https://doi.org/10.1242/dev.098178.
[51] J. de Wit, et al., Unbiased discovery of glypican as a receptor for LRRTM4 in regulating excitatory synapse development, Neuron 79 (4) (Aug. 2013) 696–711, https://doi.org/10.1016/j.neuron.2013.06.049.
[52] J.W. Fawcett, J.C.F. Kwok, Proteoglycan sulphation in the function of the mature central nervous system, Front. Integr. Neurosci. 16 (May 2022) 895493, https://doi.org/10.3389/fnint.2022.895493.
[53] V.J. Coulson-Thomas, The role of heparan sulphate in development: the ectodermal story, Int J Experimental Path 97 (3) (Jun. 2016) 213–229, https://doi.org/10.1111/iep.12180.
[54] S.P. Mutalik, S.L. Gupton, Glycosylation in axonal guidance, IJMS 22 (10) (May 2021) 5143, https://doi.org/10.3390/ijms22105143.
[55] H. Wang, et al., Chondroitin-4-sulfation negatively regulates axonal guidance and growth, J. Cell Sci. 121 (18) (Sep. 2008) 3083–3091, https://doi.org/10.1242/jcs.032649.
[56] T. Ogawa, K. Hagihara, M. Suzuki, Y. Yamaguchi, Brevican in the developing hippocampal fimbria: differential expression in myelinating oligodendrocytes and adult astrocytes suggests a dual role for brevican in central nervous system fiber tract development, J. Comp. Neurol. 432 (3) (Apr. 2001) 285–295, https://doi.org/10.1002/cne.1103.
[57] C.P. Mencio, R.K. Hussein, P. Yu, H.M. Geller, The role of chondroitin sulfate proteoglycans in nervous system development, J. Histochem. Cytochem. 69 (1) (Jan. 2021) 61–80, https://doi.org/10.1369/0022155420959147.
[58] S. Yang, et al., Antibody recognizing 4-sulfated chondroitin sulfate proteoglycans restores memory in tauopathy-induced neurodegeneration, Neurobiol. Aging 59 (Nov. 2017) 197–209, https://doi.org/10.1016/j.neurobiolaging.2017.08.002.
[59] G.A. Cabral-Pacheco, et al., The roles of matrix metalloproteinases and their inhibitors in human diseases, Int. J. Mol. Sci. 21 (24) (Jan. 2020), https://doi.org/10.3390/ijms21249739. Art. no. 24.
[60] D. Carulli, et al., Cerebellar plasticity and associative memories are controlled by perineuronal nets, Proc. Natl. Acad. Sci. USA 117 (12) (Mar. 2020) 6855–6865, https://doi.org/10.1073/pnas.1916163117.
[61] S.A. Back, et al., Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation, Nat. Med. 11 (9) (Sep. 2005) 966–972, https://doi.org/10.1038/nm1279.
[62] A.J. Hayes, J. Melrose, Aggrecan, the primary weight-bearing cartilage proteoglycan, has context-dependent, cell-directive properties in embryonic development and neurogenesis: aggrecan glycan side chain modifications convey interactive biodiversity, Biomolecules 10 (9) (Aug. 2020) 1244, https://doi.org/10.3390/biom10091244.
[63] J. Melrose, Keratan sulfate (KS)-proteoglycans and neuronal regulation in health and disease: the importance of KS -glycodynamics and interactive capability with neuroregulatory ligands, J. Neurochem. 149 (2) (Apr. 2019) 170–194, https://doi.org/10.1111/jnc.14652.
[64] C.M. Galtrey, J.W. Fawcett, The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system, Brain Res. Rev. 54 (1) (Apr. 2007) 1–18, https://doi.org/10.1016/j.brainresrev.2006.09.006.
[65] A. Hudák, A. Letoha, C. Vizler, T. Letoha, Syndecan-3 as a novel biomarker in Alzheimer's disease, IJMS 23 (6) (Mar. 2022) 3407, https://doi.org/10.3390/ijms23063407.

[66] T. Letoha, et al., Contribution of syndecans to cellular internalization and fibrillation of amyloid-β(1–42), Sci. Rep. 9 (1) (Feb. 2019) 1393, https://doi.org/10.1038/s41598-018-37476-9.
[67] W. Hofmeister, C.A. Devine, B. Key, Distinct expression patterns of syndecans in the embryonic zebrafish brain, Gene Expr. Patterns 13 (3–4) (Mar. 2013) 126–132, https://doi.org/10.1016/j.gep.2013.02.002.
[68] Q. Wang, L. Yang, C. Alexander, S. Temple, The niche factor syndecan-1 regulates the maintenance and proliferation of neural progenitor cells during mammalian cortical development, PLoS One 7 (8) (Aug. 2012) e42883, https://doi.org/10.1371/journal.pone.0042883.
[69] C. Rhiner, M.O. Hengartner, Sugar antennae for guidance signals: syndecans and glypicans integrate directional cues for navigating neurons, Sci. World J. 6 (2006) 1024–1036, https://doi.org/10.1100/tsw.2006.202.
[70] J. Ouchida, et al., Glypican-2 defines age-dependent axonal response to chondroitin sulfate, Exp. Neurol. 366 (Aug. 2023) 114444, https://doi.org/10.1016/j.expneurol.2023.114444.
[71] I. Farhy-Tselnicker, A.C.M. Van Casteren, A. Lee, V.T. Chang, A.R. Aricescu, N. J. Allen, Astrocyte-secreted glypican 4 regulates release of neuronal pentraxin 1 from axons to induce functional synapse formation, Neuron 96 (2) (Oct. 2017) 428–445.e13, https://doi.org/10.1016/j.neuron.2017.09.053.
[72] S. Lugert, et al., Glypican-2 levels in cerebrospinal fluid predict the status of adult hippocampal neurogenesis, Sci. Rep. 7 (1) (Apr. 2017) 46543, https://doi.org/10.1038/srep46543.
[73] A. Matsuyama, A.A. Kalargyrou, A.J. Smith, R.R. Ali, R.A. Pearson, A comprehensive atlas of Aggrecan, Versican, Neurocan and Phosphacan expression across time in wildtype retina and in retinal degeneration, Sci. Rep. 12 (1) (May 2022) 7282, https://doi.org/10.1038/s41598-022-11204-w.
[74] G.J. Eill, A. Sinha, M. Morawski, M.S. Viapiano, R.T. Matthews, The protein tyrosine phosphatase RPTPζ/phosphacan is critical for perineuronal net structure, J. Biol. Chem. 295 (4) (Jan. 2020) 955–968, https://doi.org/10.1016/S0021-9258(17)49907-8.
[75] A. Faissner, L. Roll, U. Theocharidis, Tenascin-C in the matrisome of neural stem and progenitor cells, Mol. Cell. Neurosci. 81 (Jun. 2017) 22–31, https://doi.org/10.1016/j.mcn.2016.11.003.
[76] S. Wiese, A. Faissner, The role of extracellular matrix in spinal cord development, Exp. Neurol. 274 (Dec. 2015) 90–99, https://doi.org/10.1016/j.expneurol.2015.05.018.
[77] S.M. Dyck, S. Karimi-Abdolrezaee, Chondroitin sulfate proteoglycans: key modulators in the developing and pathologic central nervous system, Exp. Neurol. 269 (Jul. 2015) 169–187, https://doi.org/10.1016/j.expneurol.2015.04.006.
[78] H.-C. So, et al., Identification of neuroglycan C and interacting partners as potential susceptibility genes for schizophrenia in a Southern Chinese population, Am. J. Med. Genet. 9999B (2009), https://doi.org/10.1002/ajmg.b.30961 n/a-n/a.
[79] S. Aono, et al., Expression and identification of a new splice variant of neuroglycan C, a transmembrane chondroitin sulfate proteoglycan, in the human brain, J. Neurosci. Res. 83 (1) (Jan. 2006) 110–118, https://doi.org/10.1002/jnr.20698.
[80] S. Cattaruzza, R. Perris, Proteoglycan control of cell movement during wound healing and cancer spreading, Matrix Biol. 24 (6) (Sep. 2005) 400–417, https://doi.org/10.1016/j.matbio.2005.06.005.
[81] J. Melrose, Fractone stem cell niche components provide intuitive clues in the design of new therapeutic procedures/biomatrices for neural repair, IJMS 23 (9) (May 2022) 5148, https://doi.org/10.3390/ijms23095148.
[82] A.L. Trout, et al., Perlecan domain-V enhances neurogenic brain repair after stroke in mice, Transl. Stroke Res 12 (1) (Feb. 2021) 72–86, https://doi.org/10.1007/s12975-020-00800-5.
[83] A.D. Snow, J.A. Cummings, T. Lake, The unifying hypothesis of Alzheimer's disease: heparan sulfate proteoglycans/glycosaminoglycans are key as first hypothesized over 30 Years ago, Front. Aging Neurosci. 13 (Oct. 2021) 710683, https://doi.org/10.3389/fnagi.2021.710683.
[84] B. Nguyen, G. Bix, Y. Yao, Basal lamina changes in neurodegenerative disorders, Mol. Neurodegener. 16 (1) (Dec. 2021) 81, https://doi.org/10.1186/s13024-021-00502-y.
[85] R. Nakamura, F. Nakamura, S. Fukunaga, Diverse functions of perlecan in central nervous system cells *in vitro*: functions of Perlecan in CNS Cells, Anim. Sci. J. 86 (10) (Oct. 2015) 904–911, https://doi.org/10.1111/asj.12376.
[86] A. Kerever, et al., Perlecan is required for FGF-2 signaling in the neural stem cell niche, Stem Cell Res. 12 (2) (Mar. 2014) 492–505, https://doi.org/10.1016/j.scr.2013.12.009.
[87] M. Dellett, W. Hu, V. Papadaki, S. Ohnuma, Small leucine rich proteoglycan family regulates multiple signalling pathways in neural development and maintenance, Dev. Growth Differ. 54 (3) (Apr. 2012) 327–340, https://doi.org/10.1111/j.1440-169X.2012.01339.x.
[88] Y. Xie, et al., Biglycan regulates neuroinflammation by promoting M1 microglial activation in early brain injury after experimental subarachnoid hemorrhage, J. Neurochem. 152 (3) (Feb. 2020) 368–380, https://doi.org/10.1111/jnc.14926.
[89] J.P. Huston, K. Weth, A. De Souza Silva, U. Junghans, H.W. Müller, R. U. Hasenöhrl, Facilitation of learning and long-term ventral pallidal–cortical cholinergic activation by proteoglycan biglycan and chondroitin sulfate C, Neuroscience 100 (2) (Sep. 2000) 355–361, https://doi.org/10.1016/S0306-4522(00)00270-0.
[90] S. Mizumoto, T. Kosho, S. Yamada, K. Sugahara, Pathophysiological significance of dermatan sulfate proteoglycans revealed by human genetic disorders, Pharmaceuticals 10 (4) (Mar. 2017) 34, https://doi.org/10.3390/ph10020034.
[91] K. Baerwald-De La Torre, U. Winzen, W. Halfter, J.L. Bixby, Glycosaminoglycan-dependent and -independent inhibition of neurite outgrowth by agrin: agrin's GAG chains and neurite inhibition, J. Neurochem. 90 (1) (Apr. 2004) 50–61, https://doi.org/10.1111/j.1471-4159.2004.02454.x.
[92] M. Jung Kim, S.L. Cotman, W. Halfter, G.J. Cole, The heparan sulfate proteoglycan agrin modulates neurite outgrowth mediated by FGF-2, J. Neurobiol. 55 (3) (Jun. 2003) 261–277, https://doi.org/10.1002/neu.10213.
[93] M.C. Dewan, et al., Estimating the global incidence of traumatic brain injury, J. Neurosurg. 130 (4) (Apr. 2018) 1080–1097, https://doi.org/10.3171/2017.10.JNS17352.
[94] J.-H. Yi, Y. Katagiri, B. Susarla, D. Figge, A.J. Symes, H.M. Geller, Alterations in sulfated chondroitin glycosaminoglycans following controlled cortical impact injury in mice, J. Comp. Neurol. 520 (15) (2012) 3295–3313, https://doi.org/10.1002/cne.23156.
[95] S. Tavakoli, N. Krishnan, H. Mokhtari, O.P. Oommen, O.P. Varghese, Fine-tuning dynamic cross–linking for enhanced 3D bioprinting of hyaluronic acid hydrogels, Adv. Funct. Mater. (Oct. 2023) 2307040, https://doi.org/10.1002/adfm.202307040.
[96] Y.-W. Ding, X.-W. Zhang, C.-H. Mi, X.-Y. Qi, J. Zhou, D.-X. Wei, Recent advances in hyaluronic acid-based hydrogels for 3D bioprinting in tissue engineering applications, Smart Materials in Medicine 4 (2023) 59–68, https://doi.org/10.1016/j.smaim.2022.07.003.
[97] N. George, H.M. Geller, Extracellular matrix and traumatic brain injury, J. Neurosci. Res. 96 (4) (2018) 573–588, https://doi.org/10.1002/jnr.24151.
[98] M. Ennemoser, A. Pum, A. Kungl, Disease-specific glycosaminoglycan patterns in the extracellular matrix of human lung and brain, Carbohydr. Res. 511 (Jan. 2022) 108480, https://doi.org/10.1016/j.carres.2021.108480.
[99] B.S. Elkin, M.A. Shaik, B. Morrison, Chondroitin sulfate proteoglycans contribute to brain tissue swelling behavior, in: Proceedings of the 2010 IEEE 36th Annual Northeast Bioengineering Conference (NEBEC), Mar. 2010, pp. 1–2, https://doi.org/10.1109/NEBC.2010.5458138.
[100] M. Alvarado-Velez, Immuno-suppressive hydrogels for stem cell therapy after traumatic brain injury [Online]. Available: https://smartech.gatech.edu/handle/1853/62340, Nov. 2019. (Accessed 7 January 2022).
[101] D.U. Jeong, et al., Hydrogel-mediated local delivery of dexamethasone reduces neuroinflammation after traumatic brain injury, Biomed. Mater. 16 (3) (Feb. 2021) 035002, https://doi.org/10.1088/1748-605X/abc7f1.
[102] C.M. Schneider, M.L. Jackson, S.S. Bedi, C.S. Cox, Chapter 23 - stem cells for traumatic brain injury, in: A. Atala, R. Lanza, A.G. Mikos, R. Nerem (Eds.), Principles of Regenerative Medicine, third ed., Academic Press, Boston, 2019, pp. 369–389, https://doi.org/10.1016/B978-0-12-809880-6.00023-0.
[103] B.J. Larson, M.T. Longaker, H.P. Lorenz, Scarless fetal wound healing: a basic science review, Plast. Reconstr. Surg. 126 (4) (Oct. 2010) 1172–1180, https://doi.org/10.1097/PRS.0b013e3181eae781.
[104] Z.Z. Khaing, B.D. Milman, J.E. Vanscoy, S.K. Seidlits, R.J. Grill, C.E. Schmidt, High molecular weight hyaluronic acid limits astrocyte activation and scar formation after spinal cord injury, J. Neural. Eng. 8 (4) (Jul. 2011) 046033, https://doi.org/10.1088/1741-2560/8/4/046033.
[105] C.-M. Lin, et al., Hyaluronic acid inhibits the glial scar formation after brain damage with tissue loss in rats, Surg. Neurol. 72 (Dec. 2009) S50–S54, https://doi.org/10.1016/j.wneu.2009.09.004.
[106] S.V. Kushchayev, et al., Hyaluronic acid scaffold has a neuroprotective effect in hemisection spinal cord injury, SPI 25 (1) (Jul. 2016) 114–124, https://doi.org/10.3171/2015.9.SPINE15628.
[107] J. Melrose, Glycosaminoglycans in wound healing, Bone Tissue Regen Insights 7 (Jan. 2016), https://doi.org/10.4137/BTRI.S38670. BTRI.S38670.
[108] R.J. Mckeon, A. Höke, J. Silver, Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars, Exp. Neurol. 136 (1) (Nov. 1995) 32–43, https://doi.org/10.1006/exnr.1995.1081.
[109] I. Francos-Quijorna, et al., Chondroitin sulfate proteoglycans prevent immune cell phenotypic conversion and inflammation resolution via TLR4 in rodent models of spinal cord injury, Nat. Commun. 13 (1) (May 2022) 2933, https://doi.org/10.1038/s41467-022-30467-5.
[110] E.J. Bradbury, et al., Chondroitinase ABC promotes functional recovery after spinal cord injury, Nature 416 (6881) (Apr. 2002) 636–640, https://doi.org/10.1038/416636a.
[111] A. Sharifi, et al., Sustained delivery of chABC improves functional recovery after a spine injury, BMC Neurosci. 23 (1) (Oct. 2022) 60, https://doi.org/10.1186/s12868-022-00734-8.
[112] E.J. Bradbury, L.M. Carter, Manipulating the glial scar: chondroitinase ABC as a therapy for spinal cord injury, Brain Res. Bull. 84 (4–5) (Mar. 2011) 306–316, https://doi.org/10.1016/j.brainresbull.2010.06.015.
[113] A. Rolls, et al., Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation, PLoS Med. 5 (8) (Aug. 2008) e171, https://doi.org/10.1371/journal.pmed.0050171.
[114] A. Rolls, et al., A disaccharide derived from chondroitin sulphate proteoglycan promotes central nervous system repair in rats and mice+, Eur. J. Neurosci. 20 (8) (2004) 1973–1983, https://doi.org/10.1111/j.1460-9568.2004.03676.x. Oct.
[115] A. Rolls, L. Cahalon, S. Bakalash, H. Avidan, O. Lider, M. Schwartz, A sulfated disaccharide derived from chondroitin sulfate proteoglycan protects against inflammation-associated neurodegeneration, Faseb. J. 20 (3) (Mar. 2006) 547–549, https://doi.org/10.1096/fj.05-4540fje.
[116] S. Foscarin, R. Raha-Chowdhury, J.W. Fawcett, J.C.F. Kwok, Brain ageing changes proteoglycan sulfation, rendering perineuronal nets more inhibitory, Aging 9 (6) (Jun. 2017) 1607–1622, https://doi.org/10.18632/aging.101256.

[117] H. Wang, et al., Chondroitin-4-sulfation negatively regulates axonal guidance and growth, J. Cell Sci. 121 (18) (Sep. 2008) 3083–3091, https://doi.org/10.1242/jcs.032649.
[118] S.S. Deepa, et al., Composition of perineuronal net extracellular matrix in rat brain, J. Biol. Chem. 281 (26) (Jun. 2006) 17789–17800, https://doi.org/10.1074/jbc.M600544200.
[119] T. Mikami, D. Yasunaga, H. Kitagawa, Contactin-1 is a functional receptor for neuroregulatory chondroitin sulfate-E, J. Biol. Chem. 284 (7) (Feb. 2009) 4494–4499, https://doi.org/10.1074/jbc.M809227200.
[120] E.W. Baker, H.A. Kinder, F.D. West, Neural stem cell therapy for stroke: a multimechanistic approach to restoring neurological function, Brain and Behavior 9 (3) (Mar. 2019) e01214, https://doi.org/10.1002/brb3.1214.
[121] R. De Gioia, et al., Neural stem cell transplantation for neurodegenerative diseases, IJMS 21 (9) (Apr. 2020) 3103, https://doi.org/10.3390/ijms21093103.
[122] M. Bacigaluppi, et al., Neural stem cell transplantation induces stroke recovery by upregulating glutamate transporter GLT-1 in astrocytes, J. Neurosci. 36 (41) (Oct. 2016) 10529–10544, https://doi.org/10.1523/JNEUROSCI.1643-16.2016.
[123] C. Liu, et al., Inhibition of astrocytic differentiation of transplanted neural stem cells by chondroitin sulfate methacrylate hydrogels for the repair of injured spinal cord, Biomater. Sci. 7 (5) (Apr. 2019) 1995–2008, https://doi.org/10.1039/C8BM01363B.
[124] S. Samanta, L. Ylä-Outinen, V.K. Rangasami, S. Narkilahti, O.P. Oommen, Bidirectional cell-matrix interaction dictates neuronal network formation in a brain-mimetic 3D scaffold, Acta Biomater. (Dec. 2021), https://doi.org/10.1016/j.actbio.2021.12.010.
[125] N. Hayashi, S. Miyata, Y. Kariya, R. Takano, S. Hara, K. Kamei, Attenuation of glial scar formation in the injured rat brain by heparin oligosaccharides, Neurosci. Res. 49 (1) (May 2004) 19–27, https://doi.org/10.1016/j.neures.2004.01.007.
[126] Q. Ma, et al., The blood–brain barrier accessibility of a heparin-derived oligosaccharides C3, Thromb. Res. 105 (5) (Mar. 2002) 447–453, https://doi.org/10.1016/S0049-3848(02)00050-6.
[127] B.I. Korelitz, S.C. Sommers, Responses to drug therapy in ulcerative colitis. Evaluation by rectal biopsy and histopathological changes, Am. J. Gastroenterol. 64 (5) (Nov. 1975) 365–370.
[128] S. Samanta, et al., Heparin-derived theranostic nanoprobes overcome the blood–brain barrier and target glioma in murine model, Advanced Therapeutics 5 (6) (Jun. 2022) 2200001, https://doi.org/10.1002/adtp.202200001.
[129] R.F. James, et al., Continuous infusion of low-dose unfractionated heparin after aneurysmal subarachnoid hemorrhage: a preliminary study of cognitive outcomes, J. Neurosurg. 130 (5) (May 2019) 1460–1467, https://doi.org/10.3171/2017.11.JNS17894.
[130] J.M. Simard, E.F. Aldrich, D. Schreibman, R.F. James, A. Polifka, N. Beaty, Low-dose intravenous heparin infusion in patients with aneurysmal subarachnoid hemorrhage: a preliminary assessment: clinical article, JNS 119 (6) (Dec. 2013) 1611–1619, https://doi.org/10.3171/2013.8.JNS1337.
[131] B.D. Spiess, Heparin: effects upon the glycocalyx and endothelial cells, J. Extra Corpor. Technol. 49 (3) (Sep. 2017) 192–197.
[132] S. Yini, Z. Heng, A. Xin, M. Xiaochun, Effect of unfractionated heparin on endothelial glycocalyx in a septic shock model, Acta Anaesthesiol. Scand. 59 (2) (Feb. 2015) 160–169, https://doi.org/10.1111/aas.12418.
[133] X. Li, J. Zhu, K. Liu, Y. Hu, K. Huang, S. Pan, Heparin ameliorates cerebral edema and improves outcomes following status epilepticus by protecting endothelial glycocalyx in mice, Exp. Neurol. 330 (Aug. 2020) 113320, https://doi.org/10.1016/j.expneurol.2020.113320.
[134] J. Li, J.-P. Li, X. Zhang, Z. Lu, S.P. Yu, L. Wei, Expression of heparanase in vascular cells and astrocytes of the mouse brain after focal cerebral ischemia, Brain Res. 1433 (Jan. 2012) 137–144, https://doi.org/10.1016/j.brainres.2011.11.032.
[135] B. Changyaleket, Z.Z. Chong, R.O. Dull, D. Nanegrungsunk, H. Xu, Heparanase promotes neuroinflammatory response during subarachnoid hemorrhage in rats, J. Neuroinflammation 14 (1) (Dec. 2017) 137, https://doi.org/10.1186/s12974-017-0912-8.
[136] R. Raghunathan, et al., Glycomic and proteomic changes in aging brain nigrostriatal pathway, Mol. Cell. Proteomics 17 (9) (Sep. 2018) 1778–1787, https://doi.org/10.1074/mcp.RA118.000680.
[137] R. Raghunathan, J.D. Hogan, A. Labadorf, R.H. Myers, J. Zaia, A glycomics and proteomics study of aging and Parkinson's disease in human brain, Sci. Rep. 10 (1) (Jul. 2020) 12804, https://doi.org/10.1038/s41598-020-69480-3.
[138] B.B. Holmes, et al., Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds, Proc. Natl. Acad. Sci. USA 110 (33) (Aug. 2013) E3138–E3147, https://doi.org/10.1073/pnas.1301440110.
[139] T. Kanekiyo, J. Zhang, Q. Liu, C.-C. Liu, L. Zhang, G. Bu, Heparan sulphate proteoglycan and the low-density lipoprotein receptor-related protein 1 constitute major pathways for neuronal amyloid-β uptake, J. Neurosci. 31 (5) (Feb. 2011) 1644–1651, https://doi.org/10.1523/JNEUROSCI.5491-10.2011.
[140] K. Zhang, et al., Potential application of an injectable hydrogel scaffold loaded with mesenchymal stem cells for treating traumatic brain injury, J. Mater. Chem. B 6 (19) (May 2018) 2982–2992, https://doi.org/10.1039/C7TB03213G.
[141] W.-H. Jian, et al., Glycosaminoglycan-based hybrid hydrogel encapsulated with polyelectrolyte complex nanoparticles for endogenous stem cell regulation in central nervous system regeneration, Biomaterials 174 (Aug. 2018) 17–30, https://doi.org/10.1016/j.biomaterials.2018.05.009.
[142] J. Zhong, A. Chan, L. Morad, H.I. Kornblum, G. Fan, S.T. Carmichael, Hydrogel matrix to support stem cell survival after brain transplantation in stroke, Neurorehabilitation Neural Repair 24 (7) (Sep. 2010) 636–644, https://doi.org/10.1177/1545968310361958.
[143] J. Lam, W.E. Lowry, S.T. Carmichael, T. Segura, Delivery of iPS-NPCs to the stroke cavity within a hyaluronic acid matrix promotes the differentiation of transplanted cells, Adv. Funct. Mater. 24 (44) (2014) 7053–7062, https://doi.org/10.1002/adfm.201401483.
[144] M.R. McCrary, et al., Cortical transplantation of brain-mimetic glycosaminoglycan scaffolds and neural progenitor cells promotes vascular regeneration and functional recovery after ischemic stroke in mice, Adv. Healthcare Mater. 9 (5) (2020) 1900285, https://doi.org/10.1002/adhm.201900285.
[145] Y.-T. Wei, et al., Hyaluronic acid hydrogel modified with nogo-66 receptor antibody and poly-L-lysine to promote axon regrowth after spinal cord injury, J. Biomed. Mater. Res. B Appl. Biomater. 95 (1) (Oct. 2010) 110–117, https://doi.org/10.1002/jbm.b.31689.
[146] K. Zaviskova, et al., Injectable hydroxyphenyl derivative of hyaluronic acid hydrogel modified with RGD as scaffold for spinal cord injury repair, J. Biomed. Mater. Res. 106 (4) (2018) 1129–1140, https://doi.org/10.1002/jbm.a.36311.
[147] N. Broguiere, L. Isenmann, M. Zenobi-Wong, Novel enzymatically cross-linked hyaluronan hydrogels support the formation of 3D neuronal networks, Biomaterials 99 (Aug. 2016) 47–55, https://doi.org/10.1016/j.biomaterials.2016.04.036.
[148] L. Wang, et al., Injectable hyaluronic acid hydrogel loaded with BMSC and NGF for traumatic brain injury treatment, Materials Today Bio 13 (Jan. 2022) 100201, https://doi.org/10.1016/j.mtbio.2021.100201.
[149] L.T.B. Nguyen, C.-C. Hsu, H. Ye, Z. Cui, Development of an in situ injectable hydrogel containing hyaluronic acid for neural regeneration, Biomed. Mater. 15 (5) (Jul. 2020) 055005, https://doi.org/10.1088/1748-605X/ab8c43.
[150] C. Lin, et al., *In vitro* study of human immune responses to hyaluronic acid hydrogels, recombinant spidroins and human neural progenitor cells of relevance to spinal cord injury repair, Cells 10 (7) (Jul. 2021), https://doi.org/10.3390/cells10071713, 7,.
[151] R.H. Basit, N. Tzerakis, S.I. Jenkins, D.M. Chari, *In vitro* model of traumatic brain injury to screen neuro-regenerative biomaterials, Mater. Sci. Eng. C 128 (Sep. 2021) 112253, https://doi.org/10.1016/j.msec.2021.112253.
[152] C.-C. Hsu, et al., Increased connectivity of hiPSC-derived neural networks in multiphase granular hydrogel scaffolds, Bioact. Mater. 9 (Mar. 2022) 358–372, https://doi.org/10.1016/j.bioactmat.2021.07.008.
[153] L. Karumbaiah, et al., Chondroitin sulfate glycosaminoglycan hydrogels create endogenous niches for neural stem cells, Bioconjugate Chem. 26 (12) (Dec. 2015) 2336–2349, https://doi.org/10.1021/acs.bioconjchem.5b00397.
[154] B. Newland, et al., Injectable glycosaminoglycan-based cryogels from well-defined microscale templates for local growth factor delivery, ACS Chem. Neurosci. 12 (7) (Apr. 2021) 1178–1188, https://doi.org/10.1021/acschemneuro.1c00005.
[155] C. Xu, et al., Two-dimensional-germanium phosphide-reinforced conductive and biodegradable hydrogel scaffolds enhance spinal cord injury repair, Adv. Funct. Mater. 31 (41) (2021) 2104440, https://doi.org/10.1002/adfm.202104440.
[156] J. Chen, et al., Injectable self-healing hydrogel with siRNA delivery property for sustained STING silencing and enhanced therapy of intervertebral disc degeneration, Bioact. Mater. 9 (Mar. 2022) 29–43, https://doi.org/10.1016/j.bioactmat.2021.08.003.
[157] H.H. Park, et al., Dual-functional hydrogel system for spinal cord regeneration with sustained release of arylsulfatase B alleviates fibrotic microenvironment and promotes axonal regeneration, Biomaterials 284 (May 2022) 121526, https://doi.org/10.1016/j.biomaterials.2022.121526.
[158] Y. Luo, et al., An injectable, self-healing, electroconductive extracellular matrix-based hydrogel for enhancing tissue repair after traumatic spinal cord injury, Bioact. Mater. 7 (Jan. 2022) 98–111, https://doi.org/10.1016/j.bioactmat.2021.05.039.
[159] M.I. Betancur, H.D. Mason, M. Alvarado-Velez, P.V. Holmes, R.V. Bellamkonda, L. Karumbaiah, Chondroitin sulfate glycosaminoglycan matrices promote neural stem cell maintenance and neuroprotection post-traumatic brain injury, ACS Biomater. Sci. Eng. 3 (3) (Mar. 2017) 420–430, https://doi.org/10.1021/acsbiomaterials.6b00805.
[160] C.-F.V. Latchoumane, et al., Engineered glycomaterial implants orchestrate large-scale functional repair of brain tissue chronically after severe traumatic brain injury, Sci. Adv. (Mar. 2021), https://doi.org/10.1126/sciadv.abe0207.
[161] C. Chen, et al., Collagen/heparin sulfate scaffolds fabricated by a 3D bioprinter improved mechanical properties and neurological function after spinal cord injury in rats, J. Biomed. Mater. Res. 105 (5) (2017) 1324–1332, https://doi.org/10.1002/jbm.a.36011.
[162] T.B. Ngo, B.S. Spearman, N. Hlavac, C.E. Schmidt, Three-dimensional bioprinted hyaluronic acid hydrogel test beds for assessing neural cell responses to competitive growth stimuli, ACS Biomater. Sci. Eng. 6 (12) (Dec. 2020) 6819–6830, https://doi.org/10.1021/acsbiomaterials.0c00940.
[163] X. Liu, et al., 3D bioprinted neural tissue constructs for spinal cord injury repair, Biomaterials 272 (May 2021) 120771, https://doi.org/10.1016/j.biomaterials.2021.120771.
[164] M. Tang, et al., Rapid 3D bioprinting of glioblastoma model mimicking native biophysical heterogeneity, Small 17 (15) (2021) 2006050, https://doi.org/10.1002/smll.202006050.
[165] E.M. Steel, J.-Y. Azar, H.G. Sundararaghavan, Electrospun hyaluronic acid-carbon nanotube nanofibers for neural engineering, Materialia 9 (Mar. 2020) 100581, https://doi.org/10.1016/j.mtla.2019.100581.
[166] S. Song, X. Liu, J. Huang, Z. Zhang, Neural stem cell-laden 3D bioprinting of polyphenol-doped electroconductive hydrogel scaffolds for enhanced neuronal

differentiation, Mater. Sci. Eng. C (Jan. 2022) 112639, https://doi.org/10.1016/j.msec.2021.112639.
[167] M. Modo, S.F. Badylak, A roadmap for promoting endogenous in situ tissue restoration using inductive bioscaffolds after acute brain injury, Brain Res. Bull. 150 (Aug. 2019) 136–149, https://doi.org/10.1016/j.brainresbull.2019.05.013.
[168] K. Ioannidis, et al., 3D Reconstitution of the neural stem cell niche: connecting the dots, Front. Bioeng. Biotechnol. 9 (2021) 705470, https://doi.org/10.3389/fbioe.2021.705470.
[169] Z. Xu, S. Liu, M. Liang, H. Yang, C. Chang, Biomaterials based growth factor delivery for brain regeneration after injury, Smart Materials in Medicine 3 (Jan. 2022) 352–360, https://doi.org/10.1016/j.smaim.2022.04.001.
[170] D. Hachim, T.E. Whittaker, H. Kim, M.M. Stevens, Glycosaminoglycan-based biomaterials for growth factor and cytokine delivery: making the right choices, J. Contr. Release 313 (Nov. 2019) 131–147, https://doi.org/10.1016/j.jconrel.2019.10.018.
[171] C.-Y. Yang, et al., Neural tissue engineering: the influence of scaffold surface topography and extracellular matrix microenvironment, J. Mater. Chem. B 9 (3) (Jan. 2021) 567–584, https://doi.org/10.1039/D0TB01605E.
[172] P.A. Walczak, P. Perez-Esteban, D.C. Bassett, E.J. Hill, Modelling the central nervous system: tissue engineering of the cellular microenvironment, Emerg Top Life Sci 5 (4) (Oct. 2021) 507–517, https://doi.org/10.1042/ETLS20210245.
[173] A.S. Hoffman, Hydrogels for biomedical applications, Adv. Drug Deliv. Rev. 64 (Dec. 2012) 18–23, https://doi.org/10.1016/j.addr.2012.09.010.
[174] N. Hlavac, M. Kasper, C.E. Schmidt, Progress toward finding the perfect match: hydrogels for treatment of central nervous system injury, Materials Today Advances 6 (Jun. 2020) 100039, https://doi.org/10.1016/j.mtadv.2019.100039.
[175] P. Hen Lin, Q. Dong, S. Yian Chew, Injectable hydrogels in stroke and spinal cord injury treatment: a review on hydrogel materials, cell–matrix interactions and glial involvement, Materials Advances 2 (8) (2021) 2561–2583, https://doi.org/10.1039/D0MA00732C.
[176] N. Ghane, M.-H. Beigi, S. Labbaf, M.-H. Nasr-Esfahani, A. Kiani, Design of hydrogel-based scaffolds for the treatment of spinal cord injuries, J. Mater. Chem. B 8 (47) (Dec. 2020) 10712–10738, https://doi.org/10.1039/D0TB01842B.
[177] C.I. Gama, et al., Sulfation patterns of glycosaminoglycans encode molecular recognition and activity, Nat. Chem. Biol. 2 (9) (Sep. 2006), https://doi.org/10.1038/nchembio810, 9.
[178] Z.Z. Khaing, S.K. Seidlits, Hyaluronic acid and neural stem cells: implications for biomaterial design, J. Mater. Chem. B 3 (40) (Oct. 2015) 7850–7866, https://doi.org/10.1039/C5TB00974J.
[179] S. An, S. Choi, S. Min, S.-W. Cho, Hyaluronic acid-based biomimetic hydrogels for tissue engineering and medical applications, Biotechnol Bioproc E 26 (4) (Aug. 2021) 503–516, https://doi.org/10.1007/s12257-020-0343-8.
[180] G. Tejeda, A.J. Ciciriello, C.M. Dumont, Biomaterial strategies to Bolster neural stem cell-mediated repair of the central nervous system, CTO (Jun. 2021) 1–15, https://doi.org/10.1159/000515351.
[181] E. Bellotti, A.L. Schilling, S.R. Little, P. Decuzzi, Injectable thermoresponsive hydrogels as drug delivery system for the treatment of central nervous system disorders: a review, J. Contr. Release 329 (Jan. 2021) 16–35, https://doi.org/10.1016/j.jconrel.2020.11.049.
[182] J. Samal, T. Segura, Injectable biomaterial shuttles for cell therapy in stroke, Brain Res. Bull. 176 (Nov. 2021) 25–42, https://doi.org/10.1016/j.brainresbull.2021.08.002.
[183] P. Zarrintaj, et al., Injectable cell-laden hydrogels for tissue engineering: recent advances and future opportunities, Tissue Eng. (Jun. 2021), https://doi.org/10.1089/ten.tea.2020.0341.
[184] M. Cadena, et al., 3D bioprinting of neural tissues, Adv. Healthcare Mater. 10 (15) (2021) 2001600, https://doi.org/10.1002/adhm.202001600.
[185] G. Decante, J.B. Costa, J. Silva-Correia, M.N. Collins, R.L. Reis, J.M. Oliveira, Engineering bioinks for 3D bioprinting, Biofabrication 13 (3) (Apr. 2021) 032001, https://doi.org/10.1088/1758-5090/abec2c.
[186] A. Burnstine-Townley, Y. Eshel, N. Amdursky, Conductive scaffolds for cardiac and neuronal tissue engineering: governing factors and mechanisms, Adv. Funct. Mater. 30 (18) (2020) 1901369, https://doi.org/10.1002/adfm.201901369.