

## Astrogliosis: A review of the astrocytic mechanisms, imaging modes, and treatments in spinal cord injury patients

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**Abstract:** Astrocytes are cells in the central nervous system (CNS) that are responsible for many things, such as maintaining blood brain barrier (BBB), regulating synapses in the spinal cord, and responding to spinal cord injury (SCI). Astrogliosis, the astrocytic response to spinal cord injuries (SCIs), helps repair CNS damages by regulating different protein filaments, thus limiting axonal growth. Former studies that were demonstrated through laser capture microdissection and immunohistochemistry (IHC) helped to identify important genes involved in experimental therapies for SCIs. Additionally, there are potential clinical treatments options for SCIs such as hydrogels, mesenchymal stem cells and steroids. Increased imaging modalities indicate that excessive astrogliosis can have adverse effects. These imaging techniques include positron emission tomography (PET), magnetic resonance imaging (MRI), and two-photon laser-scanning microscopy (TPLSM). These techniques illuminate greater details of the astrocytic response to SCIs. Despite these findings, astrogliosis is not well understood by the research community. Many of the studies presented in this literature review are experimental attempts to understand the mechanisms of astrogliosis in SCIs. This literature review aims to summarize the methods of each study in visualizing the mechanisms of astrogliosis and how they play a role in SCIs. Furthermore, this paper is aimed to comprehensively bridge the developments in the treatment for SCI patients based on innovative imaging modalities. Compared to prior studies, this review utilizes more recent understandings of the astrogliosis mechanisms to highlight insights into targeted developments, both clinically and preclinically. Some limitations of this literature review include the limited studies on astrogliosis and its impact on SCIs. Nonetheless, there is ongoing potential in the search for treatments for SCIs.

**Keywords:** Astrogliosis, Astrocytosis, Reactive astrogliosis, Spinal cord injury

### Introduction: astrocytes, astrogliosis, and spinal cord insult

Astrocytes are cells within the central nervous system (CNS) that are responsible for the formation and maintenance of the blood brain barrier (BBB). They accomplish this by producing intrinsic factors that regulate the signals that pass

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through the synapses. During repolarization, neurons release  $K^+$  in the synaptic cleft, which are then taken in by the astrocytes from their increased  $K^+$  channels [1-2]. The  $K^+$  ions are then released into other parts of the body [3]. For example, the release of  $K^+$  in blood vessels surrounding the brain allows for the maintenance of the BBB [4]. Additionally, astrocytes are also responsible for the synaptic function in the spinal cord, which maintains its structure and function. Astrocytes also regulate the glutamate transmission and calcium influx and efflux in the synapses [5-7]. They contribute to the regulation of optimal pH in the CNS through the cellular respiration of glucose molecules into protons and water. The excess protons produced help to maintain the pH of the system [8]. The role of astrocytes can also be demonstrated based on the consequences of removing the functional protein in astrocytes that provide its function. Liedtke et al. [5, 9] stated that without the functional protein found in astrocytes, a vast array of complications potentially arise such as impaired myelination, hydrocephalus and motor deficits. There are different classes of astrocytes that fulfill different functions for the spinal cord and body [10, 11]. For example, type 1 astrocytes are responsible for fibroblast growth signaling, while type 2 astrocytes are responsible for the response to spinal cord injury (SCI) [12-14].

The terms astrogliosis, reactive astrogliosis, and astrocytosis are used interchangeably in the current literature to describe the structural and functional changes in astrocytes that take place in response to CNS perturbations including both injury and disease. Although these astrocytic modifications play a well-established role in determining the secondary outcomes following a CNS insult, their contributions to cellular repair and mitigation of these aforementioned outcomes are comparatively covered less but cannot be underestimated [15]. With regards to baseline physiology, astrocytes or astroglia are essential to CNS function, including maintenance as reflected by the overabundance of this cell type in humans and many other mammals [16]. These neural cells, broadly speaking, play a role in homeostasis including cellular and network homeostasis, molecular homeostasis, systemic homeostasis, organ homeostasis and metabolic homeostasis [17]. Moreover, the heterogeneity of astrocyte function is not only enabled by the abundance of cells, but also by the morphological diversity manifested in the protein expression of a variety of receptors, channels and transporters [18]. Reactive astrogliosis to pathology similarly presents a spectrum of mechanisms resulting in a continuum comprising both loss and gain of function [19]. Reactive astrogliosis is not an “all-or-nothing” process; ramifications range from short-term, reversible regulatory changes to CNS specific gene expression with subsequent cell and tissue damage to seemingly more irreversible structural and functional tissue rearrangements including glial scar formation [20].

Reactive astrogliosis to CNS trauma, CNS pathology (stroke, infection/inflammation), and neurodegenerative diseases (Alzheimer’s, Huntington’s, Parkinson’s) similarly

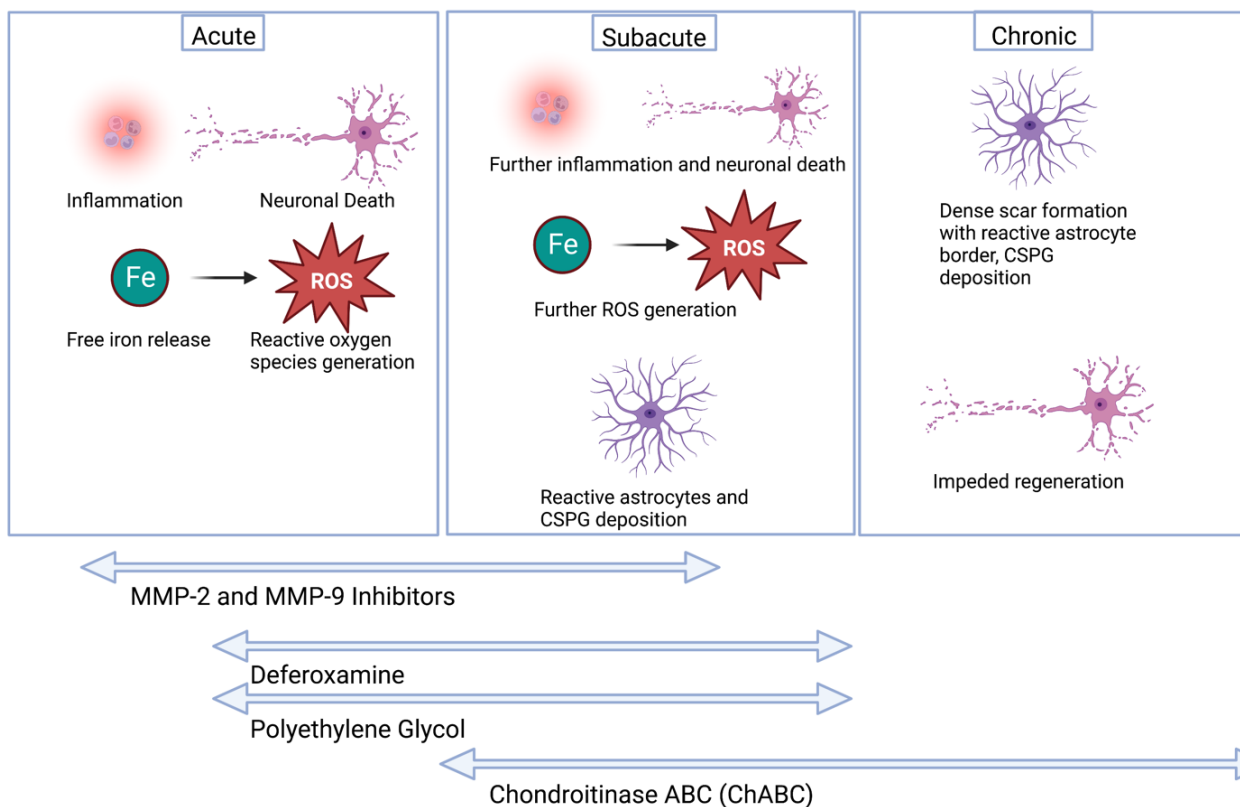
occur in a temporal manner through established phases. Overlap in these cellular changes between a wide array of neurological conditions has encouraged researchers over the years to postulate a complex role for astrogliosis and glial scarring that includes both a damaging and protective role, referred to as a “dual role” [21, 22]. In evidence for the latter contribution, Gu et al. [23, 24] were able to demonstrate that reactive astrocyte ablation at the site of SCI in a mouse model resulted in widespread impairment to physiological repair mechanisms. Moreover, increased inflammation was found to be coupled with significant cell and tissue damage in the aforementioned study. As the timeline for specific SCIs progress, dynamic interactions between the local glial cells (oligodendrocytes, astrocytes, microglia, and ependymal cells) along with the extracellular matrix (ECM) contribute to the development of glial scarring [25, 26] (Figure 1). With regards to SCI (direct or indirect), the initial cellular response (within seconds to hours) is largely mediated by inflammatory cytokines including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1) and interleukin-6 (IL-6) [27, 28]. This inflammation cascade is considered a secondary injury to the primary SCI. Furthermore, cytokine signaling results in recruitment of not only immune cells but also glial cells such as astrocytes to the damaged site [29]. As a byproduct of the localized infiltration, tissue edema around the area also builds up [27]. Astrocyte recruitment is additionally enhanced via signaling from the proximal injured cells [30, 31]. Moreover, the available free iron increases as a byproduct of blood infiltration around the spinal cord. The free ion is then able to readily generate reactive oxygen species (ROS) by way of the Fenton reaction [32]. ROS and cytokine signaling drive the inflammatory process towards an apoptotic state [33].

As astrogliosis occurs to mitigate and possibly repair CNS damage in the body, different molecular changes arise. Astrogliosis occurs with increased responses and regulation of different filament proteins such as glial fibrillary acidic proteins (GFAP), vimentins and chondroitin sulfate proteoglycans (CSPG) [34-36]. As a result, these molecules among others limit axonal growth. Studies have indicated that CSPGs contain glycosaminoglycan (GAG), which specifically contributes to a significant inhibition of axonal growth. Bradbury et al. [35] demonstrated that removal of GAG from CSPGs subsequently impaired axonal inhibition, suggesting an active role of GAG in astrogliosis and neuronal growth. These GAG chains repeatedly consist of multiple disaccharide units. After the initial astrocyte reactivity, glial scar formation begins within one or two days after injury [37]. During this astrocyte transformation process, reactive astrocytes transform into scar-forming cells and become so proliferative and dense enough to create a border-like structure around the injury site [25, 38]. Glial scar formation occurs with the interaction of the astrocytic  $\beta 1$  integrin receptor ( $\beta 1R$ ) and collagen [39-41]. In order to prevent scars from triggering, an anti- $\beta 1$  integrin antibody ( $\beta 1Ab$ ) is administered. Researchers have discovered that

administering  $\beta$ 1Ab into the body affects the position of the microglia [42, 43]. Moreover, the development of these scar-forming astrocytes depends on the N-cadherin and integrin signaling pathway, which underlies neuronal and glial (including astrocytes) neurite extension [38, 44]. This multitude of signaling interactions enables reactive astrocytes to morphologically transform into entities that are more suitable for lesion coverage, particularly scar formation. Over several weeks, the glial scar develops into a more defined, compact structure. The accompanying astrocytic changes have been demonstrated to proceed through a JAK-STAT3 dependent pathway. The function of the glial scar is not well understood. As previously stated, there is evidence that the structure and the interaction processes surrounding it play both an inhibitory and a protective role. Physically, the glial scar provides a barrier. However, a chemical component responsible for neurotoxicity and subsequent inhibition of axonal growth also presents an obstruction to recovery [45]. On the other hand, reactive astrocytes and glial scar forming astrocytes have been proven to be equipped with anti-inflammatory responsibilities [24]. Overall, SCI is followed by a diverse set of signaling cascades and interactions that heavily rely

on the inherent changes to astrocytes located at the level of lesion. As detailed, the roles of glial cells involved in SCI dynamically proceed through the timeframe of secondary injury development. Clinically, treatments aimed at SCI recovery must consider temporal resolution as a factor in targeting specific aspects of the lesion. Due to the vast array of molecular signaling that occurs within the SCI timeframe, several possible downstream target molecules such as CSPGs can be specifically tested regardless of the initial upstream developments. Preclinically, the plasticity potential of astrocyte subsequent to SCI presents an intriguing source of molecular manipulations to prevent SCI from ever fully developing.

The present literature review aims to characterize the obstacles presented by reactive astrogliosis in the context of SCI. We intend to outline the spinal cord changes mediated by astrocytosis and discuss how advanced imaging can be used to detect the aforementioned changes. We also plan to elaborate on the current preclinical and clinical state of treatment options for SCI. Lastly, we will detail the limitations of overcoming reactive astrogliosis and therefore SCI in addition to the direction of future prospects in this area of study.



**Figure 1.** Illustrated timeline progression of spinal cord injury (SCI) from immediately to weeks after initial damage. Images demonstrate localized activity of reactive astrocytosis (acute), glial scar formation (subacute), and glial scar maturation (chronic). Molecules depicted at the bottom have been shown to contribute to cellular and extracellular matrix (ECM) repair in addition to mitigation of damage such as oxidative stress

## Preclinical phase treatment

Currently, therapeutics for astrogliosis are being developed within both the preclinical and clinical realms. This section discusses the mechanisms that target the pathophysiology of astrogliosis associated with SCIs at the molecular level. As mentioned above, the role of particular stages of astrogliosis has been elucidated as crucial steps in the pernicious consequences of glial scar formation. An appreciable number of therapies have been developed in reshaping the trajectory of astrocyte formation.

In 2017, Hara et al. [38] used laser capture microdissection and immunohistochemistry (IHC) to identify specific genes that may be potentially significant in astrogliosis. They also utilized a genome wide expression analysis to determine genes that are uniquely upregulated in the microenvironment of the naïve spinal cord compared to the injured spinal cord at different time points after injury. The former identified genes are particular to the three described stages of astrocyte progression in SCIs – NA, RA and SA. For example, SA abundantly expresses the calcium-dependent adhesion molecule N-cadherin, among others [46]. In the latter, a marked increase (> 5-fold) was identified in several gene types within the injured spinal cord system rather than in specific cell types. This was observed in approximately 5% of genes within the set analyzed. The first of these categories was an ECM component, type 1 collagen genes (Col1L), which include both Col1a1 and Col1a2 [38]. This was consequentially confirmed via IHC analysis. Acting upon this knowledge, plating experiments were conducted to qualify the nuances of this dynamic, and to determine how exactly Col1L genes may influence astrocyte development. RAs were performed and evaluated under two conditions – Col1 coated and non Col1 coated dishes. The coated dishes displayed less retraction (suggesting less attenuation of scar tissue), and significantly increased staining for N-cadherin. As previously mentioned, N-cadherin was a uniquely expressed biomarker in SAs compared to NAs and RAs, indicating that Col1 genes may play a role in enhancing N-cadherin contacts within RAs and promote their development into SAs. Regarding the potential therapies based on these observations, the same group developed beta-1 antibodies that bind collagen-binding integrins, thereby blocking formation of SA and promoting axonal regeneration. Therefore, targeted monoclonal antibody therapy may be a viable form of treatment for astrogliosis if more genes are discovered and targeted.

Overall, the role of ECM related molecules in the progression of RA development is beginning to be well acknowledged in the study of SCIs [47-49]. In processes such as high-throughput RNA sequencing, upregulated SCI genes were identified to develop focus on specific molecules, similar to the Hara group. Experimental therapies have employed both inhibitory and activating

approaches to promote axonal regeneration. As such, periostin (POSTN), an ECM molecule, has been sought after as an inhibitory target. Both genetic deletion and pharmacological inhibition of POSTN suppressed scar growth, particularly by preventing the proliferation of pericytes [49]. Activating measures included the stimulation of Epac2, which is involved in the regrowth of axonal tissue [50]. Additionally, targeting Epac2 indicates a route to therapy that does not directly involve modulation of astrocyte development. The initial theory for this therapeutic stemmed from previous experiments that exhibited gradient dependent attraction of axons to Epac agonists, similar to their attraction for cAMP [51-53]. Additionally, in studies, introduction of Epac agonists has also resulted in signs of neuronal healing such as myelination and neurite outgrowth [54]. cAMP modulation has been acknowledged as an effective pathway to promote axonal growth, though its systemic presence prevents targetable remedies without predisposing to unwanted toxicities. Therefore, alternative pathways, such as those described, have focused attention on addressing cAMP related mechanisms by indirectly targeting the associated molecules. Indeed, Belmar et al. [50] explored the potential utility of this method in various applications of Epac2 to dorsal root ganglion (DRG) samples. When incubated with Epac2 agonist, a significant chemoattractant response was observed in the growth cones of the DRGs compared to controls. More astoundingly, the inhibitory effects of CSPGs on neurite outgrowth (both dendritic and axonal) were seemingly overcome by the presence of Epac2 agonist. Rat cortical neurons grown with Epac2 agonist exhibited a mean neurite to neuron ratio of 32.1, compared to 8.7 in untreated samples. To complement the possible implications of this, Epac2 agonist was introduced into DRG neuron cultures containing mature, inhibitory astrocytes, a significant source of CSPGs. Overall, the mean proportion of growth cones that grew over astrocytes (rather than retracting) was 45.7% compared to controls. In the discussion of CSPGs, many other endeavors have also revealed further insight into the granular mechanisms of CSPGs and their interaction with other molecules that can be utilized as forms of targeted therapy. From previous work highlighting the role of leukocyte common antigen related (LAR) and protein tyrosine phosphatase-sigma (PTPsigma), Dyck et al. [55-56] developed selective blockers for these, thereby generating a pro-inflammatory M1 response to a healing M2 response. The relationship between LAR and PTPsigma upregulation of CSPG was then employed to promote a regenerative response to SCI. These set of experiments further elucidate the specific pathways that may be targeted in consideration of potential therapeutics for astrogliosis.

Alternative outlets of therapy have been developed with the intent to mitigate the proinflammatory effects of specific astrocyte phenotypes, namely the A1 phenotype described in the background. Vismara et al. [57] developed



a vehicle for drug delivery, reminiscent of nanoparticle (NP) technology. Their variation of this was the nanogel (NG), which boasts a greater colloidal stability in addition to longer durations of cargo retention. The experiments within this study focused on delivery of Rolipram, an anti-inflammatory drug that acts on NF- $\kappa$ B. NG delivery loaded with Rolipram reduced the levels of inducible nitric oxide synthase (iNOS) and Lcn2 in pro-inflammatory treated cultures (i.e. TNF- $\alpha$  and IL- $\alpha$ ) [58]. These pro-inflammatory cytokines have been implicated in the transformation of the inflammatory astrocyte state (A1 phenotype). Kinesthetic assessment was conducted in the form of locomotive performance of treated mice. Similar observations were noted of which SCI mice treated with Rolipram loaded NG displayed significantly higher levels of locomotor performance. These experiments suggest that focusing therapy on the downstream inflammatory effects of A1 may also possess therapeutic utility.

## Clinical phase treatment

Several novel treatments have shown promise in ameliorating SCI sequelae. One of these is hydrogels, an analog of the extracellular matrix of a soft tissue [59]. In their systematic review and meta-analysis of animal models, Ayar et al. [59] found notable improvements in the pathophysiological outcomes of SCI after treatment with hydrogels. One such finding includes the potential of hydrogels to improve motor function after SC. The authors conducted a meta-analysis of Basso, Beattie, and Bresnahan scores and found that there were statistically significant improvements in functional capacity after either injection or implantation of hydrogels [59]. Of note, there was high heterogeneity among the studies which was addressed through subsequent subgroup analysis [59]. The etiology of the SCI was strongly indicative of motor function recovery [59]. In particular, hemisection and transection injuries were found to have the highest beneficial effects of hydrogel therapy [59]. Of note, hydrogels administered after SCI were found to be effective in reducing inflammation and glial scar formation, which in turn promoted axon regeneration [59].

Similar to hydrogels, other potential forms of novel therapeutics have also shown promise in reducing SCI inflammation and its pathophysiological consequences. In particular, mesenchymal stem cells (MSC) and their paracrine activity show promising beneficial effects [60]. IL-10, TGF- $\beta$ , PGE-2 are among the many anti-inflammatory molecules secreted by MSCs [60]. Furthermore, it has been shown that MSCs stimulate cells to produce antioxidant enzymes [60]. This could result in a protective cellular effect and thereby reduce the oxidative stress to which the cells are exposed during inflammation. As such, the anti-inflammatory and pro-antioxidant properties of MSCs could reduce the damage experienced by neural tissues [60]. Other potentially

beneficial properties of MSCs include gliosis inhibition and neural regeneration [60]. Similarly, neurotrophin-derived therapies have shown potential clinical efficacy in gliosis inhibition, while also having superior pharmacokinetics compared to traditional neurotrophins [61]. One of which is BB14, a small molecule agonist of neurotrophin receptors [61]. BB14 promoted TrkA activity to the same extent as native nerve growth factor (NGF), which subsequently showed promise in the treatment of astrogliosis [61].

Currently, there is a large amount of clinical disagreement regarding the use of high-dose steroids to reduce SCI inflammation. Liu et al's [62] meta-analysis attempted to resolve this in their meta-analysis. Their study evaluated the efficacy of high-dose methylprednisone intervention in patients with SCI. The results showed no statistical difference in neurologic recovery and higher rates of secondary adverse complications in patients receiving high-dose methylprednisone [62]. Liu et al's [62] study highlights the need for research initiatives tailored to improve SCI treatment regimens. As such, the encouraging therapeutic benefits of the aforementioned novel therapies should be further explored in future studies in order to improve the pathophysiological outcomes of SCI.

## Imaging modalities

Astrocytes are a type of glial cells that are found in the CNS and play an important role in the health and function of neurons. However, in response to different CNS insults or injuries, astrocytes undergo structural and functional changes that include their activation and subsequent proliferation, leading to the formation of a glial scar through a process called astrogliosis. The glial scar is believed to function as a barrier that isolates damaged or injured areas of the CNS in order to prevent further injury. While astrogliosis is generally understood as a protective response to CNS injury, excessive or prolonged astrogliosis can have negative effects, such as promoting damaging inflammatory responses and inhibiting axonal regrowth. Currently, many efforts have been made to understand the role of astrogliosis in neuronal injury and recovery. Given the link of astrogliosis to a range of neurological pathologies such as traumatic brain injury, stroke, Alzheimer's disease and multiple sclerosis, there is a need to understand the molecular mechanisms behind astrogliosis in order to provide effective diagnosis and therapeutic treatment.

Given the dual role of astrocytes in neurophysiology, emerging advanced imaging modalities are playing an increasingly crucial role in understanding the disease process of astrogliosis. One of the emerging imaging modalities that have been used in the study of reactive astrocytes is positron emission tomography (PET). PET relies on the use of an injectable radioactive tracer that

travels through the body and is absorbed by specific cells or tissues, where it releases a type of radiation called positrons, which can then be detected by a PET scanner. The PET scanner is then able to create a detailed 3D image of the body by mapping and quantifying the number of positrons emitted from the tracer. PET scans are commonly used as a tool to assess the activity of the CNS and to search for neuropathology. Thus, PET is a valuable tool in the study of astrogliosis. In addition, the development and emergence of new PET tracers have contributed to the understanding of the underlying mechanisms of astrogliosis in neuropathology [63]. Harada et al. [63] also discussed the potential benefits of using *in vivo* PET imaging in the study of reactive astrogliosis and using it as a tool to assess therapeutic efficacy as well as disease progression. PET studies have shown altered astrocyte metabolism in the brains of patients with Alzheimer's disease, as well as increased astrocyte activity in glioma.

Another salient imaging modality is multidimensional magnetic resonance imaging (MRI). MRI takes advantage of a strong magnetic field and radio energy to produce a detailed image of the body. Thus, making it a safe and non-invasive assessment tool. Multifunctional MRI refers to the use of advanced techniques that not only can provide a high-resolution detailed 3D image of the brain structure but also can provide crucial information about its function like brain activity and connectivity. Novel use of multifunctional MRI combined with artificial intelligence has been able to detect that specific microstructural and morphological changes to astrogliosis resulting in a distinct multidimensional MRI spectral signature, which has crucial implication in the diagnosis and treatments for individuals with different neuropathologies [64].

Besides, to expand our understanding behind the complex biology surrounding astrocytes and their behaviors, imaging modalities have been used as a way to study astrogliosis and glial scar formation after injury using *in vivo* animal models. One study used two-photon laser-scanning microscopy (TPLSM) to follow the response of GFP-labeled astrocytes [65]. TPLSM is a powerful imaging technique that allows for live cell imaging providing an accurate observation on cells behavior with minimal damage. TPLSM uses two photons to selectively excite fluorescent molecules in a defined volume of tissue. The ability of TPLSM to capture images of living cells makes it highly beneficial for studies of the brain, as it provides functional data about individual neurons and their connections. TPLSM can also detect changes in neuronal activity over time. Thus, the use of TPLSM modality provides a great way to understand and characterize the heterogenic behavior and function of astrocytes and the role of astrogliosis in glial scar formation. The imaging modality of live cells provided an evidence against what was previously believed the migratory function of astrocytes towards the site of injury. Instead, astrocytes close to the site of injury become

hypertrophic, thereby upregulating GFAP, a known marker for astrocyte reactivity and reactive astrogliosis [66].

On the other hand, functional magnetic resonance imaging (fMRI) is a commonly used non-invasive imaging modality in the assessment of neural activity associated with changes of blood flow that takes advantage of the correlation between neuronal activation and cerebral blood flow. To assess the neuronal activity of the spinal cord caudal on the site of injury in patients with SCI, a study used fMRI of the spinal cord (spinal fMRI) in order to assess the functional activity and treatment efficacy. The study demonstrated the ability of fMRI to detect neuronal activity of the spinal cord as a way for functional assessment in patients with SCI [67]. Furthermore, another study demonstrated the benefits of using imaging modalities in the setting of SCI. This study used Filter-probe diffusion imaging to assess the functional and histological outcomes following SCI. The study showed the prognostic benefits of combining imaging modalities with acute functional scores [68]. Thus, both fMRI and filtered-probe diffusion imaging demonstrate the importance of advanced imaging modalities for the early detection and diagnosis of neuronal injuries as well as provide a valuable resource in the study and understanding of neuropathologies, such as astrogliosis and glial scar formation.

In summation, emerging imaging modalities are proved to be a valuable asset in the study of astrogliosis by helping to advance the understanding of the complex role that astrocytes function and morphology play in various neuropathologies in the CNS.

## Current landscape, future directions, and limitations

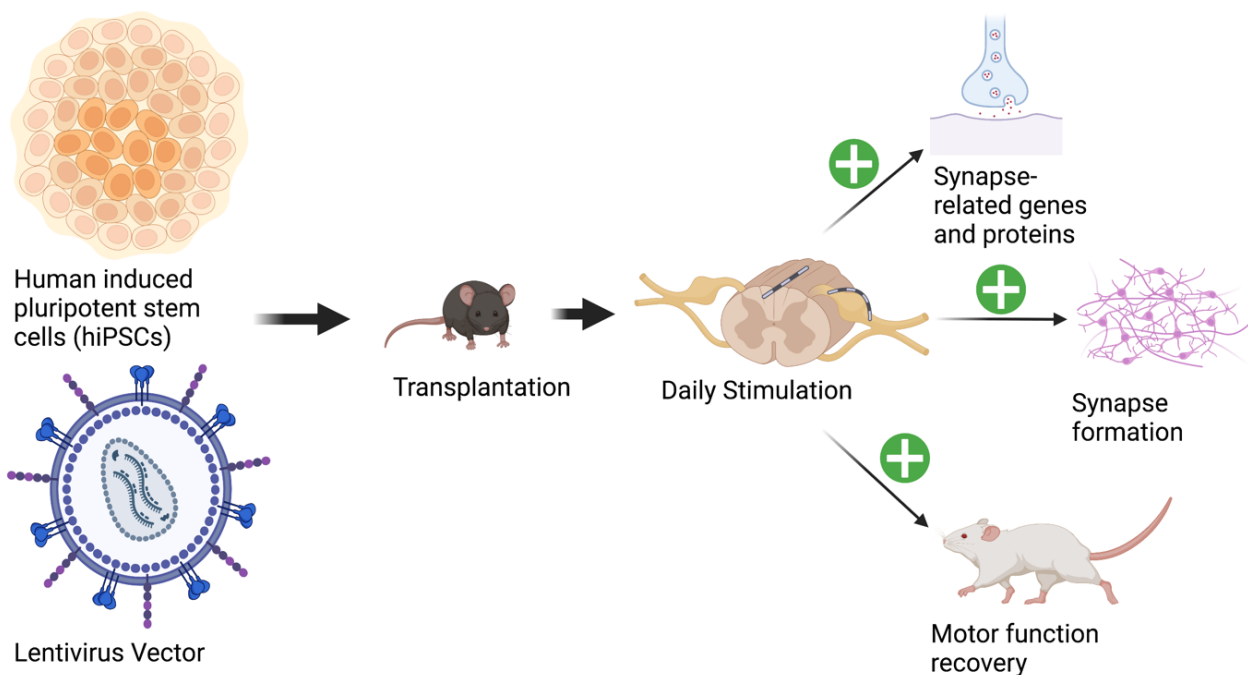
Promising frontiers in the therapeutic attenuation of astrogliosis include stem cell transplantation, gene therapy, ECM remodeling and electromagnetic stimulation.

Currently, human pluripotent stem cells (hiPSC) are on the forefront of stem cell replacement. Kawai et al. [69], identified genetic stimulation of hiPSC-derived neural cells as stimulator of synapse-related genes and proteins, neuron-neuron interactions, and cell activity. Human embryonic stem cells are harvested from the inner layer of an early blastocyst. This provides a relative ease of access and a vast differentiation potential. The literature suggests that future efforts based on stem cell may incorporate combined cell and drug therapy with pluripotent stem-cell derived neural cells [70]. This method may create a permissive environment for regeneration, maximizing growth properties while minimizing inhibitory properties. A prominent challenge will remain modulating this interplay to selectively “fine-tune” regeneration.

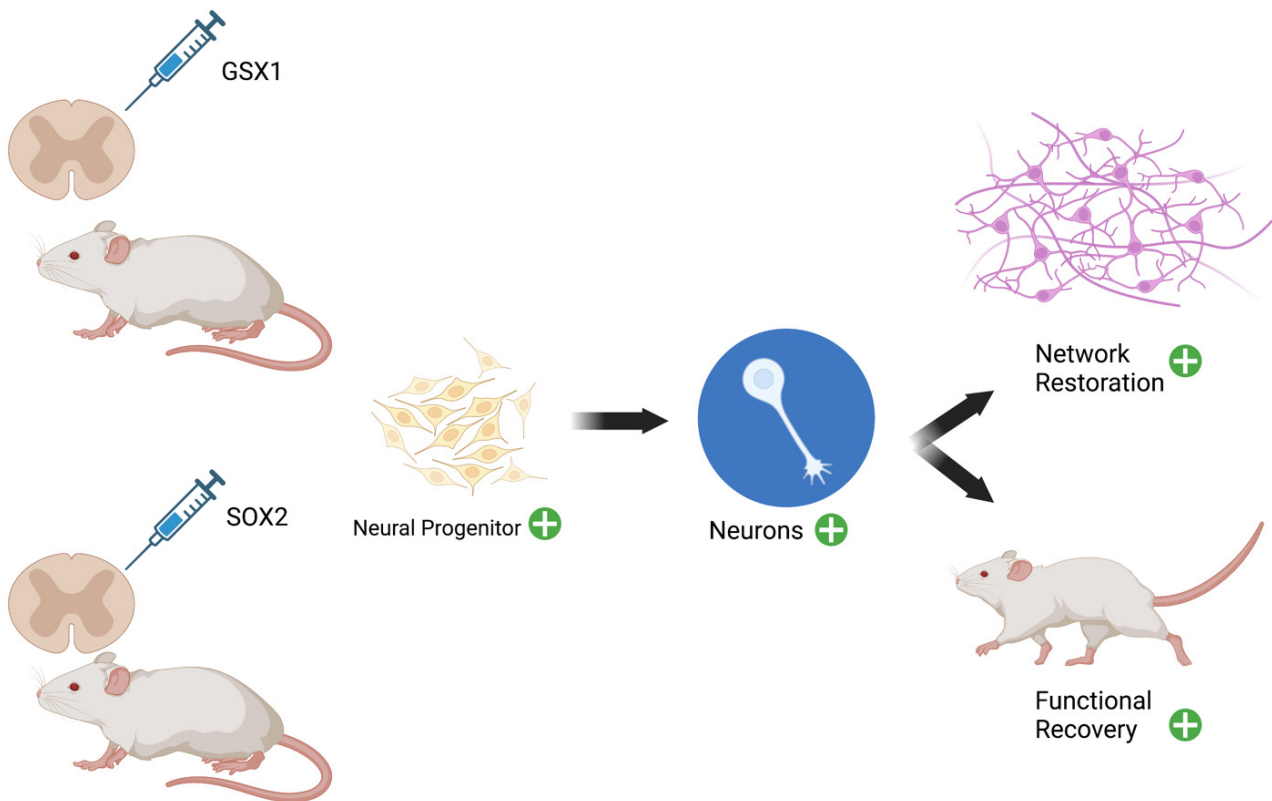
Several studies have identified gene therapy as a promising therapeutic potential. Tai et al. [71] suggested

ectopic SOX-2 expression may stimulate the network expansion and scar reduction by NG2 glial cells. Reprogramming these cells may unmask latent neuro-regenerative potential and promote functional recovery. Patel et al. [72] also identified Gsx1 expression as stimulating excitatory interneurons and inhibiting inhibitory interneurons in chronic-phase SCI. Gsx1 expression was associated with reduced reactive astrogliosis and glial scar formation. These changes were associated with improved locomotion in mice. Although Gsx1 therapy both enhanced the generation of excitatory interneurons and limited the formation of glial scar, no functional recovery was observed. This suggests there may be additional factors that limit the impact of astrogliosis on recovery. Similar limitations were reported by Tai et al. [71], in modulating SOX-2 expression. Gene therapies also raise some important considerations due to their use of viral vectors. Adenovirus is a popular vector which is unique for allowing transient expression of a transgene, coupled with targeted delivery to the neuromuscular junction. This proves beneficial in SCI in which genomic changes beyond the repair period are not required, and in which sensitive anatomic regions not requiring repair may

be left unaffected. Despite these advantages, adenovirus is strongly immunogenic and is classified as a level 2 risk group by the NIH [73]. Lentivirus is useful in SCI due to its ability to transfect both dividing and non-dividing cells [74]. As many cell types are injured in SCI, these make for a versatile mechanism. Because this is an HIV-associated virus, therefore, safety concerns arise. The risk of a replication competent virus is marginal but present. There is also a more pronounced risk of insertional mutagenesis [75]. Adeno associated viruses may be the most promising viral vector in SCI repair. This is the most commonly used vector due to its low immunogenicity, low risk of insertional mutagenesis, and ability to transfect both dividing and non-dividing cells [76]. Cross packaging with different capsids can also allow for targeted and judicious delivery [77]. Its primary limitation is its inability to accommodate large genes [78]. Despite these limitations, gene therapy remains advantageous in its selectivity in both targeting particular cells through using cell-specific promoters and being capable of dually silencing and stimulating gene expression (Figures 2 and 3).



**Figure 2.** Stem cell therapy schematic in mouse spinal cord injury (SCI) model. Viral vector transfection into the mouse model provides the stimulation necessary to neural regeneration and functional recovery



**Figure 3.** Gene therapy schematic in spinal cord injury (SCI) mouse model promoting reduced reactive astrogliosis/glial scar formation and locomotor functional recovery. Previous studies have found efficacy in particular gene expression including GSX1 and SOX2

Another therapeutic frontier is ECM modulation. Chondroitinase ABC (ChABC) is a promising ECM target which cleaves GAG side chains on CSPG core proteins [79]. CSPGs have been implicated in preventing regeneration after SCI [79]. Breaking down of these components may improve neural plasticity. Garcia-Alias et al. [80] observed the improvements in limb function following immediate administration. A meta-analysis by Sharifi et al. [81], demonstrated a successful using of hydrogel scaffolds to deliver Chondroitinase ABC. Delivery of this enzyme was shown to improve functional recovery following SCI in animal models. ECM manipulation is not without its risks. Dissolving glial scar components may result in aberrant sprouting, and uncontrolled plasticity may lead to dysreflexia or neuropathic pain [79]. Other limitations include short half-life of enzymes and immunogenic effects if bacterial enzymes are employed. This field is still developing and has yet to be translated into clinical trials.

A new interneuron subtype has been discovered, which stimulates recovery of walking following SCI. The stimulation of these neurons promoted recovery of walking, while ablation prevented recovery [82]. Future works should aim to better understand the specific cytological and recovery characteristics of newly

discovered and recovery-specific neuron types. This method is highly practical due to its cost effectiveness and relative safety.

Additional limitations of treating spinal cord reactive gliosis are that the understanding of astrocytes at this stage is highly limited for lacking discernment about the cytological characteristics, markers and regenerative abilities of astrocytes [83]. There is also limited understanding of the induction process which transforms reactive astrocytes into scar-forming astrocytes. Additionally, although astrogliosis can be simulated cytologically at a small scale, the in-vivo models to study this process are lacking [84]. Currently, single-cell approaches are being primarily used in this field [83, 85]. Although they are useful for better understanding cytological characteristics, they remain limited in their clinical use. Current in-vitro trauma simulation models involving animal or synthetic models demonstrate a recovery potential, but still fall short of ensuring success in-vivo [84]. Another limitation is regenerative potential of certain therapies may depend on the nature of SCI (type of injury, severity, location). Finally, the current literature primarily focuses on studying regeneration. As such, the impact of attenuating astrogliosis on rehabilitation--a multisystem process factoring in joints, tendons, connective



tissue and the quantity and quality of functional recovery--requires further study. Lastly, other non-modifiable factors may impact recovery after SCI. For example, age has been found to be associated with increased inflammation following SCI [79].

## Conclusion

SCI affects an estimated 450,000 people in the United States alone. Afflicted people range in severity, however they are all subject to sensory and motor deficits as SCI is a serious medical condition [86]. Although extensive research aimed at both treating SCI and preventing its further secondary damage has been conducted, there has not been a mainstream success in the functional repair and recovery of patients from a medicinal point of view [29]. A significant barrier to the development of more robust medical treatment options in SCI cases stems from the complex nature of SCI under normal physiological or pathophysiological conditions. Secondary injury to SCI is multifactorial, including damage from physical obstruction, neurotoxicity, inflammation, oxidative stress and edema. Overall damage results in ischemic changes and ultimately, death of cell or tissue. The timeline of secondary SCI entails a diverse set of cellular and molecular changes, particularly in regard to astrocytic changes. These developments proceed through the stages of initial astrocyte recruitment and reactivity and then glial scar formation/maturation. This review has attempted to provide an understanding of treatment (preclinical and clinical) and imaging options on the basis of the accompanying cellular and molecular changes that underlie secondary SCI. Although functional repair/recovery and in vivo success for SCI have been limited, there has been some success in targeting specific molecules, particularly CSPGs/ECM molecules. Furthermore, there is a future potential to advantageously use astrocytic plasticity during SCI as a means to promote autologous regeneration. However, the nuanced capacities of these cells are not fully understood at this time.

## Abbreviations

$\beta$ 1Ab –  $\beta$ 1 integrin antibody;  
 $\beta$ 1R –  $\beta$ 1 integrin receptor;  
BBB – blood brain barrier;  
ChABC – chondroitinase ABC;  
CNS – central nervous system;  
Col1L – type 1 collagen genes;  
CSPG – chondroitin sulfate proteoglycan;  
DRG – dorsal root ganglion;  
ECM – extracellular matrix;  
fMRI – functional magnetic resonance imaging;  
GAG – glycosaminoglycan;

GFAP – glial fibrillary acid protein;  
hiPSC – human pluripotent stem cells;  
IHC – immunohistochemistry;  
IL-1 – interleukin-1;  
IL-6 – interleukin-6;  
iNOS – inducible nitric oxide synthase;  
LAR – leukocyte common antigen-related protein;  
MRI – magnetic resonance imaging;  
MSC – mesenchymal stem cell;  
NG – nanogel;  
NGF – nerve growth factor;  
NP – nanoparticle;  
PET – positron emission tomography;  
POSTN – periostin;  
PTPsigma – protein tyrosine phosphate-sigma;  
ROS – Reactive oxygen species;  
SCI – spinal cord injury;  
SCIs – spinal cord injuries;  
TNF- $\alpha$  – tumor necrosis factor-alpha;  
TPLSM – two-photon laser-scanning microscopy

## Authors' contributions

Conceptualization: BLW, Alexander N, Andrew N;  
Investigation: Alexander N, Andrew N, NG, RA, HL;  
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## Conflicts of interest

All authors declared that there are no conflicts of interest.

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## Institutional review board statement

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