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1 **Moving beyond the **glial** scar for spinal cord repair**

2

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24

25

26 **Abstract**

27

28 Traumatic spinal cord injury results in severe and irreversible loss of function. The injury
29 triggers a complex cascade of inflammatory and pathological processes, culminating in the
30 formation of a scar. While traditionally referred to as a glial scar, the spinal injury scar in fact
31 comprises multiple cellular and extracellular components. This multidimensional nature should
32 be considered when aiming to understand the role of scarring in limiting tissue repair and
33 recovery. In this Review we discuss recent advances in understanding the composition and
34 phenotypic characteristics of the spinal injury scar, the oversimplification of defining the scar
35 in binary terms as good or bad, and the development of therapeutic approaches to target scar
36 components to enable improved functional outcome after spinal cord injury.

37

38

39 **Introduction**

40

41 It is estimated that more than **27 million** people worldwide are living with long-term disability
42 following a spinal cord injury¹, 90% of which result from trauma and 10% as a secondary
43 consequence of disease. Following traumatic spinal cord injury, death of spinal neurons at the
44 injury level leads to paralysis of denervated musculature and the disruption of long spinal
45 tracts leads to loss of sensation and motor control — injured descending axonal projections
46 can no longer innervate motor neuron pools below the injury level, and injured ascending
47 axonal projections can no longer provide appropriate transmission of sensory information to
48 the brain. This results in the dysregulation of multiple organ systems throughout the body and
49 a devastating loss of function². **Despite recent progress in developing experimental**
50 **therapeutics aimed at enhancing tissue repair and neuroplasticity**, there are still no effective
51 pathology-modifying or regenerative treatments available to spinal injured individuals^{3,4}.
52 Following diagnosis and acute medical interventions to stabilize clinical status, the outcome
53 is largely determined by the management of resultant symptoms, and rehabilitation to
54 maximize residual neural function².

55

56 The lack of repair following spinal cord injury is due to both cell intrinsic factors and the
57 extrinsic injury environment. Neurons of the adult mammalian central nervous system (CNS)
58 have low intrinsic regenerative ability due to a lack of growth driving signals, and suboptimal
59 availability or arrangement of subcellular machinery to enable growth cone reformation and
60 axonal elongation⁵. Experimental efforts to unlock regeneration potential at the level of the
61 cell body of the neuron have focussed on growth signalling pathways, individual regeneration-

62 associated genes, and transcriptional and epigenetic networks⁶⁻⁹. Regenerative strategies
63 have also aimed to increase synthesis and transport of materials required for growth and
64 modulate axonal cytoskeletal dynamics to promote elongation or branching^{6,7,10-12}.

65

66 The injury microenvironment also plays a key role in limiting functional repair after spinal cord
67 injury, an important component of which is the formation of a scar. *As a healing response, the
68 scar acts to spatially contain and isolate damage. However, reactive injury processes fail to
69 restore spinal tissue architecture and composition, pathology continues to propagate and the
70 tissue within and around the scar remains dysfunctional. Moreover, within the scar there are
71 extracellular factors which themselves actively inhibit restoration of function. These act acutely
72 and chronically to prohibit compensatory changes in neurons which, perhaps if overcome,
73 could transform the scar into a more effective repair process which both isolates damage and
74 generates an environment in which injury could be surmountable.*

75

76 Here we review recent literature regarding the composition and role of the *spinal injury scar*,
77 including processes leading to scar formation and maintenance, the cellular and extracellular
78 components of the scar, and how these interact with other mediators of tissue pathology. *We
79 discuss the complexities of the scar and its seemingly opposing roles, often classified in an
80 overly simplistic manner as good or bad, and finally we discuss the potential for therapeutic
81 targeting of the scar to achieve functional repair of the injured spinal cord.*

82

83

84 **Scar formation and maintenance**

85 *Tissue scarring in periphery and CNS*

86 Injury to any tissue results in a healing response, the purpose of which is firstly to curtail
87 damage and restore homeostasis and then, where possible, to restore tissue and organ
88 function. Inflammation, tissue reformation (involving cell proliferation and/or migration) and
89 tissue remodelling are conserved repair processes, although their success in restoring
90 function varies across different tissues. *A scar consists of the cells and extracellular matrix
91 (ECM) formed as the result of attempted wound repair. In many organs, the formation of a
92 scar is associated with a resolution phase and restoration of key functions of the tissue. The
93 healed tissue may not directly recapitulate the pre-injury state but it regains some ability to
94 execute its original function¹³. However, the process of tissue scarring in the CNS is more
95 complex than for many other tissues, and is associated with chronic non-resolving pathology.*

96

97 *Disease and injury to the CNS is almost always accompanied by some degree of reactive
98 gliosis, inflammation and scarring. Scar tissue and associated deposition of ECM molecules*

99 such as chondroitin sulfate proteoglycans (CSPGs, discussed in more detail below) has been
100 reported in humans and experimental animal models following traumatic brain injury¹⁴ and
101 stroke¹⁵, as well in neurodegenerative disorders such as Alzheimer's Disease¹⁶, and disorders
102 with a predominantly demyelinating and inflammatory pathology such as multiple sclerosis^{17,18}.
103 However, despite the occurrence of reactive tissue changes and scarring in several other CNS
104 pathologies, spinal cord injury represents a particularly striking example where wound repair
105 is inefficient and injury-induced pathological changes are insurmountable.

106

107 There are several likely contributing factors to these regional and injury-specific differences in
108 CNS scarring. Cell types involved in scarring in different CNS regions are phenotypically
109 different¹⁹. There are also differences in the levels of neuroinflammation and astrocyte
110 activation after brain and spinal cord trauma, with increased expression of inflammatory
111 cytokines and potentially damaging leukocytes²⁰⁻²² and more abundant and widespread
112 astrogliosis in spinal cord injuries compared to brain injuries^{14,20}. There are also differences
113 in ECM composition and distribution between brain and spinal cord pathologies²³⁻²⁵. In this
114 review we specifically focus on scarring following spinal cord injury, and discuss the dynamic
115 cellular and extracellular interactions that culminate in a hostile scar environment with limited
116 capacity for repair.

117

118 *Defining the spinal injury scar*

119 The scar that forms after a spinal cord injury is generally considered to have two distinct
120 components: the lesion core, which is primarily composed of stromal-derived fibroblasts and
121 inflammatory immune cells, and the lesion border, or penumbra, which surrounds the core
122 and is primarily composed of hypertrophic astrocytes²⁶⁻²⁸. The term glial scar has historically
123 been used to describe the astrocyte border component of the scar²⁹⁻³¹, although some
124 investigators use the term more widely to reflect the entire lesion including both glial and non-
125 neural components³². Other distinctions have been made between the glial scar and the
126 fibrotic scar³³, and specific components of the lesion (the core, the astrocyte border, and
127 surrounding tissue) have recently been referred to as lesion-related tissue compartments³¹.
128 While these distinctions are valid, and scar components undoubtedly become spatially
129 compartmentalized in a chronic scar, these components are nonetheless interlinked, and
130 evidence suggests that there may be temporal dependence and bi-directional cross talk
131 between them. **Furthermore, the scar should not be considered as an isolated component of**
132 **spinal injury pathology since it is spatio-temporally shaped by processes of inflammation and**
133 **tissue and matrix remodeling.** Here we use the term spinal injury scar, which encompasses
134 both cellular and extracellular components **across the lesion core, lesion border and**

135 **surrounding penumbra** (Figure 1). Below, we discuss the events after the initial spinal cord
136 trauma that contribute to spinal injury scar formation and maintenance

137

138 *Acute signaling events post-injury*

139

140 The majority of spinal cord injuries feature a contusive component, where compromised spinal
141 canal shape or volume causes physical deformation of spinal cord tissue² (rarer presentations
142 include sharp penetrating trauma to the spinal cord, or purely ischemic lesions following
143 vascular compromise). Tissue deformation transmits shearing and compressive forces on
144 axons and blood vessels and initiates a cascade of pathological processes. Below, we discuss
145 these processes, focusing on changes occurring immediately after the initial spinal cord
146 trauma up to one day post injury. **These acute post-injury events signal the beginning of the
147 injury cascade, which culminates in chronic pathology and scarring (summarized in Figure 2).**

148

149 Vascular trauma leads to hemorrhage, and accumulating blood sera increases tissue colloid
150 osmotic pressure, causing local edema and swelling³⁴. This damage, along with vasospasm
151 of spared vessels, leads to tissue ischemia. ATP released from damaged or metabolically-
152 compromised cells acts on purinergic receptors to induce microglial chemotaxis towards **the
153 injury zone** to protect against spread of damage³⁵⁻³⁸. **ATP gradients are also propagated via
154 connexins**³⁹. Oligodendrocytes, oligodendrocyte precursor cells (OPCs), microglia and
155 astrocytes express a heterogeneous mix of P2 receptor subtypes, and respond reactively to
156 increasing levels of ATP following trauma^{40,41}.

157 Subsequently, tissue reperfusion induces further oxidative stress, glutamate release and
158 death of neighbouring neurons and glia via excitotoxicity⁴². ATP release, dramatic loss of
159 cellular and extracellular ionic homeostasis and excessive calcium levels results in the
160 activation of calpains, phospholipase A₂ and lipoxigenase. This is followed by the generation
161 of bioactive lipid mediators and free radicals⁴³. Progressive oxidation of fatty acids in cell
162 membranes and myelin (lipid peroxidation) occurs. Furthermore there is feed-forward
163 propagation of the injury and **cell reactivity**, as bioactive mediators potentiate ATP-mediated
164 calcium increases in glia⁴⁰ and additional blood vessel endothelial cell damage results in an
165 expanding zone of hemorrhagic necrosis⁴⁴.

166

167 Concomitantly, hemostasis is the first stage of wound healing: endothelial cell trauma results
168 in platelet adhesion and activation, the coagulation cascade, and thrombin-mediated
169 conversion of fibrinogen to fibrin, to form a clot. The onset of hemostasis⁴⁵ is associated with
170 reactive changes in resident glia and represents a potent inflammatory stimulus. Platelets
171 themselves are an abundant source of inflammatory peptides and protein mediators and

172 release cytokines, chemokines and eicosanoids which readily communicate with resident
173 spinal cord cells and non-resident leukocytes. These signals cause rapid neutrophil infiltration
174 within an hour (peaking within 24 hours), which secrete MMP9, a type IV collagenase which
175 acts on basement membranes to further permeabilise the blood brain barrier³⁴. Furthermore,
176 cellular and extracellular factors (which are rapidly induced as a result of trauma, injury
177 expansion and necrosis) constitute host-derived danger signals⁴⁶. Damage associate
178 molecular patterns (DAMPs) are sterile inflammatory stimuli (such as ATP, HMGB1, IL33)
179 which activate prototypical pathogen recognition receptors (TLRs, NLRs, signalling via MAPK,
180 NFkB) to induce secretion of proinflammatory cytokines and chemokines from both neurons
181 and glia, compounding reactive gliosis and acting to recruit circulating immune responders<sup>47-
182 50</sup>.

183 184 *Non-resolving pathology*

185 Non-resolving pathology results in incomplete tissue repair and formation of a scar. Each cell
186 type that contributes to non-resolving pathology, and its respective phenotype, is inherently
187 linked to the environment it finds itself in. This environment constitutes a milieu of other
188 resident and non-resident cells, the signals they transmit and the biochemical and biophysical
189 properties of the extracellular environment in which they reside. Below, we first discuss cellular
190 and extracellular changes occurring in the sub-acute period, from days to several weeks after
191 the initial spinal cord trauma, and their contribution to the spinal injury scar. **Cellular and
192 extracellular components of the spinal injury scar in the acute and chronic phases of spinal
193 cord injury are depicted in Figure 1.**

194 195 **Cellular components of the scar**

196 ***Astrocytes***

197 **Astrocytes become reactive following spinal cord injury. The degree of reactivity is influenced
198 by a number of cell-surface receptors for DAMPS and proinflammatory cytokines and
199 chemokines⁵¹, and ranges from temporary changes in gene expression and cell morphology
200 to significant hypertrophy, spatial rearrangement and proliferation (collectively termed
201 astrogliosis)^{52,53}. Additionally, diverse astrocyte subsets and phenotypes may exist following
202 spinal cord injury (Box 1). Astrocytes are also known to dynamically switch from reactive to
203 quiescent when transposed from injured to naïve spinal cord⁵⁴. Reactive astrocytes densely
204 populate the borders of the injury epicentre, hypertrophy and strongly upregulate the
205 expression of intermediate filament proteins such as GFAP, nestin and vimentin^{53,55,56}. This
206 corresponds with elongation and extension of overlapping processes (unlike parallel and radial
207 processes found throughout normal CNS tissue architecture) and the organization of
208 astrocytes into a barrier-like structure⁵⁷.**

209

210 This barrier is reinforced by proliferation and organization of a local astrocyte population at the
211 injury border, thought to be mediated via STAT-3 dependent signalling^{58,59} and leucine zipper-
212 bearing kinase (LZK, MAP3K13) expression⁵⁶. Ependymal cell-derived astrocyte-like progeny
213 contribute to this population of astrocytes in certain spinal injury models⁶⁰, though evidence
214 suggests this is of minor contribution following contusive injuries⁶¹. Tight linking of astrocytes
215 at the injury borders is associated with reformation of the glia limitans and the containment of
216 immune cells and fibroblast-like cells within the injury epicenter, via ephrin-mediated cellular
217 adhesion^{62,63}. Thus, a population of reactive astrocytes act to spatially isolate damage and
218 fibrosis from spared tissue.

219

220 An overlapping population of astrocytes are further associated with the chronic maintenance
221 of this structure. These are referred to as scar forming astrocytes⁶⁴. Though astrocytes alone
222 are not responsible for the formation of a scar, they are major cellular players activated and
223 maintained during post-injury pathology, inflammation and tissue and matrix remodelling.
224 Alongside other cells and extracellular factors, astrocytes shape the scar cellular and
225 extracellular milieu sub-acute and chronically.

226

227 *Fibroblast-like cells*

228 Fibroblasts are ubiquitous in peripheral connective tissues and organs and are the principal
229 generators of stroma, including the ECM. By contrast, under normal conditions within the CNS,
230 fibroblast-like cells are mostly associated with the vasculature, and contribute only to the basal
231 laminae. However, injury to the spinal cord induces a significant fibroblast response which
232 produces matrix components. These matrix components may inhibit neural regeneration
233 directly, and promote prolonged tissue remodelling via interaction with inflammatory cells
234 (detailed below). These stromal elements become spatially compartmentalised by
235 surrounding reactive astrocytes to form the fibrotic core of the spinal injury scar.

236

237 Fibroblasts proliferate from meningeal cells if the dura is compromised^{27,33} and can derive from
238 perivascular cells in rats⁶⁵ and mice⁶³ following contusion injury. There is some evidence that
239 fibroblast-like stromal cells derive from pericytes; the PDGFR β ⁺ Glast⁺ perivascular cell
240 population, termed type A pericytes, proliferate in response to injury and contribute to fibrotic
241 scarring⁶⁶. Preventing Glast1+ cell proliferation leads to failure of wound sealing, exacerbated
242 lesion volume and decreased matrix deposition⁶⁶, whereas moderate reduction of pericyte-
243 derived fibrosis was found to reduce scar pathology and confer functional recovery⁶⁷.
244 However, whether this is a truly separate population or whether it overlaps with known cell
245 types (such as Glast+ astrocytes in the glia limitans) is unclear.

246

247 An increase in type-1 pericytes (distinct from those described above) has also been described,
248 following a non-contusive dorsal funiculus lesion using a nestin-GFP/NG2-DsRed transgenic
249 mouse line⁶⁸. However, NG2 is also a marker of OPCs, Schwann cells and macrophages
250 following spinal cord injury⁶⁹. Thus, there is some ambiguity as to the response of pericytes
251 following injury, although collectively there is evidence that fibroblast-like cells are derived
252 from a perivascular PDGFR β + origin^{63,66,70}.

253

254 *Oligodendrocyte precursor cells*

255 NG2+ OPCs become reactive **after spinal cord injury**, have a significant proliferative capacity
256 **and are spatially intermingled with other reactive glia at the injury border^{71,72}**. Two main
257 **contributions of OPCs within the spinal injury scar environment have been described, with**
258 **seemingly opposing roles**. OPCs contribute to remyelination, either by oligodendrogenesis or
259 through differentiation into remyelinating Schwann cells⁷³⁻⁷⁵, and also hypertrophy and
260 increase expression of NG2, **a proteoglycan thought to mediate entrapment of neurons⁷⁶**.
261 **However, the roles of OPCs in the spinal injury scar have been obscured experimentally by**
262 **overlap of markers with other cells. Alongside NG2, PDGFR α , two ganglioside antigens, and**
263 **a cyclic nucleotide phosphodiesterase (also thought to be expressed in microglia), OPCs also**
264 **express the traditional astrocyte marker GFAP, and may differentiate into a de novo population**
265 **of astrocytes in the scar^{72,77}**. Additionally, both PDGFR α and NG2 are also thought to be
266 **expressed by at least one type of perivascular/pericyte-type cell⁷¹**. The use of fate mapping
267 transgenic mouse lines, such as those expressing Cre recombinase under control of the
268 **PDGFR α promoter/enhancers^{75,73,74,78}**, alongside inclusion/exclusion of specific markers, will
269 further define their contribution to remyelination **after spinal cord injury**.

270

271 *Resident microglia and innate and adaptive immune cells*

272 By 24 hours after spinal cord injury, blood-derived monocytes are recruited into the lesion.
273 Upon extravasation, DAMPs and the associated reactive and inflammatory environment,
274 shape their differentiation phenotype. Meanwhile, resident microglia retract cellular processes
275 and become morphologically indistinguishable from infiltrated monocyte-derived
276 macrophages. Until recently, microglia and macrophages were only distinguishable using
277 relative gene expression of CD45 (macrophages being defined as CD11b⁺, CD45^{high} and
278 microglia CD11b⁺, CD45^{low}) or chimeric models. Specific, or enriched, markers for microglia
279 have now been discovered, including transmembrane protein 119 (Tmem119)⁷⁹, P2ry12 and
280 Fc receptor-like S (FCRLS)⁸⁰. **Tracking resident microglia via a genetic strategy has shown**
281 **that spared microglia proliferate and repopulate the lesion core alongside monocyte-derived**

282 **macrophages**³⁸ and recent availability of Tmem119 reporter mice^{81,82} will further elucidate the
283 role of microglia in spinal injury scarring.

284

285 A monocyte-derived or microglia-derived macrophage phenotypic spectrum exists, from pro-
286 inflammatory (M1, secreting TNF α , IL-1 β , IL-6, IL-12) to pro-repair (M2, secreting IL-10, IL13).
287 Following spinal cord injury, there is initially a mixed M1/M2 response⁸³. The release of pro-
288 inflammatory cytokines at the injury site further mobilizes resident and blood-derived cells to
289 phagocytose debris^{84,85} and affects the phenotype of other nearby resident cells..

290

291 The adaptive immune system also plays a role. After spinal cord injury, the recruitment of $\gamma\delta$ T
292 cells, and production of proinflammatory IFN γ occurs within 24 hours following injury⁸⁶. Other
293 adaptive immune system components are recruited by 7 days, and contribute to non-resolving
294 trauma-induced autoimmunity⁸⁷. Leukocytes present DAMP-derived antigens (such as MBP)
295 to T and B-cells. B cells, in turn, may present antigens to T-cells, triggering their expansion.
296 Furthermore, B-cells differentiate into plasma cells synthesizing auto-antibodies, further
297 fuelling a feed-forward immune response⁸⁸.

298

299 Unlike conditions in which successful wound healing occurs, there is no effective resolution to
300 cellular recruitment and inflammation after spinal cord injury. Monocyte or microglia-derived
301 macrophages remain in the injured spinal cord indefinitely⁸⁹. Macrophages maintain 45% of
302 peak activation months after injury⁹⁰ and their phenotype does not undergo the switch from
303 pro-inflammatory to pro-repair associated with the next phases of wound-healing in other
304 organ tissues⁹¹. The early arginase1+ (M2-like) differentiating infiltrating population is not
305 maintained⁸³ and the spectrum of innate immune cell activation phenotype is predominately
306 M1 polarized. Adaptive immunity is non-resolving, whereby lymphocytes remain indefinitely in
307 the spinal injury scar.

308

309 *Interaction between cell types*

310 The response of astrocytes, OPCs, microglia and infiltrating innate and adaptive immune cells
311 is continually influenced by one another and the tissue environment (Figure 2 depicts the time
312 course of reactive resident and non-resident cell recruitment and activation, and their cross-
313 linked interactions which lead to the chronic spinal injury scar). There are multiple direct and
314 indirect cellular interactions mediated by cytokines and chemokines which underlie this
315 (alongside interactions which occur via ECM components, discussed below). Perivascular
316 astrocyte endfeet are an integral part of the endothelial blood spinal cord barrier, and thus
317 astrocytes are in a position to regulate the magnitude of leukocyte recruitment. Astrocyte

318 expression of Socs3⁹² or NFκB⁹³ increases monocyte infiltration to the lesion epicentre. In
319 addition, resident and infiltrated microglia/macrophages express a number of receptors for
320 proinflammatory chemokines and cytokines released by reactive astrocytes (such as IL-6,
321 IL1β, CCL2), contributing to a cell signalling environment which potentiates M1 polarization.
322 In turn, astrocytes express receptors for a number of inflammatory mediators released by
323 immune cells (including IFNγ, IL6, IL1β, TNFα), inducing extensive astrocyte reactivity and
324 astrogliosis^{53,94}. Thus, there is an intimate link between astrocytes and resident and infiltrating
325 immune cells during formation of the spinal injury scar. Astrocyte-fibroblast interactions have
326 been shown to spatially compartmentalise the fibrotic core⁶² and recent evidence suggests
327 that microglia may provide an additional interface between these cells, which the authors term
328 the microglial scar³⁸.

329

330 In addition to direct cellular cross-talk, almost all parenchymal cells express receptors for a
331 multitude of signalling molecules present in the external injury microenvironment and indirectly
332 affect cell activation and phenotype of surrounding cells. Within this, there are canonical
333 regulators. For example, abolishing Wnt signalling in OPCs has been shown to reduce
334 monocyte accumulation and astrocyte hypertrophy⁹⁵. Furthermore, neurons themselves are
335 directly contacted by cells in a manner which inhibits reestablishment of neuronal connectivity.
336 Proinflammatory ED1/CD68+ macrophages induce axonal dieback upon contact^{96,97}, NG2+
337 OPCs mediate neuronal entrapment⁷⁶, and perivascular Glast 1+ cells are also directly
338 contacted by stalled axons⁶⁷.

339

340 Importantly, the reactive cellular responses after spinal cord injury are not effectively resolved
341 and many aspects are maintained chronically. Macrophages retain activity long-term with
342 maintained M1 like characteristics. Glia continue to be reactive in regions of tissue spared by
343 injury and in areas remote from the site of trauma⁹⁸, partly in response to Wallerian
344 Degeneration^{99,100}. Proximal to the lesion, glia remain hypertrophic, forming a compacted
345 astroglial scar border in which spared tissue is permanently isolated from a zone of unresolved
346 pathology, fibrosis and tissue loss. This zone is not effectively repopulated by neurons or glia
347 and, in most mammalian species, develops into a chronic cystic cavity (Figure 1).

348

349 **Extracellular components of the scar**

350 The CNS ECM is rich in glycoproteins and proteoglycans. Hyaluronan forms a backbone for
351 the attachment of tenascins and sulphated proteoglycans, stabilised by link proteins. This is
352 arranged either diffusely in the interstitial space or more densely assembled around the cell
353 soma of particular neuronal subtypes (as perineuronal nets), or around axonal nodes of
354 ranvier or synaptic boutons. These structures confer neural stability, localizing molecules such

355 as CSPGs, which effectively restrict large-scale plasticity following a critical period in
356 development¹⁰¹. Following injury, resident glia and also stromal cells, which do not normally
357 contribute parenchymal matrix, begin to contribute matrix components, and extracellular
358 DAMPs are present in both sub-acute and chronic phases.

359

360 A vast number of ECM molecules undergo differential regulation following spinal cord injury
361 (for a large scale validation see⁵⁰) and many of these play a role in neuroprotection or
362 spontaneous repair and are not refractory to recovery. Fibrous matrix forms a seal or tissue
363 bridge between retracting lesioned parenchyma. This is particularly apparent in injuries where
364 spinal tissue is rendered non-continuous, such is the case following hemisection or
365 transection. Fibroblast-derived collagenous matrix is a major component³³ (and *in vivo*
366 ablation of fibroblasts compromises tissue integrity following injury)⁶⁶. Basal laminae is
367 restored via matrix deposition of collagen VI, nidogen, fibronectin and laminin, which are
368 traditionally neuronal-growth permissive molecules. However, in the context of spinal cord
369 injury, such ECM molecules are also implicated in pathological tissue remodelling or
370 inflammation. For example, fibronectin, matrix glycoprotein tenascin C and hyaluronan
371 fragments are also endogenous TLR ligands and represent DAMPs⁴⁸.

372

373 *Extracellular components fuel fibrosis and scarring*

374 Initial and expanding secondary pathology generates a large amount of cellular debris,
375 sustained by longer-term Wallerian degeneration, oligodendrocyte apoptosis and
376 demyelination. The presence of debris, and its breakdown products, supports an ongoing
377 foam-cell-like macrophage phenotype¹⁰² where ineffective phagocytosis and lipid processing
378 means extracellular stimuli are maintained⁸⁵ and are presented to adaptive immune cells,
379 which contributes to a non-resolving auto-immune response to injury⁸⁷. Thus, the extracellular
380 environment is undergoing both chronic inflammation and glial reactivity, associated with
381 aberrant tissue remodelling and matrix deposition. There is increasing understanding as to
382 how these processes are intertwined.

383

384 A recent study finds that perivascular PDGFR β + cells (which the authors of the study term
385 pericytes) upregulate expression of the ECM molecule periostin, which in turn upregulates
386 TNF α expression from infiltrating monocyte-macrophages and leads to proliferation of
387 PDGFR β + cells, type-I collagen deposition and fibrosis⁷⁰. Perivascular-derived type-1
388 collagen has also recently been implicated in linking fibrosis and astrogliosis, where an N-
389 cadherin dependent interaction between extracellular type-1 collagen and astrocytes was
390 found to induce scar-forming astrogliosis in mice⁵⁴. Thus, there is an expanding role for

391 perivascular Col1 α 1-cell derived fibrotic matrix described following contusion injury in mice⁶³.
392 PDGFR β +, fibronectin-rich fibrotic matrix deposition is also observed in rats in the peripheral
393 rim of the cavity and outlining blood vessels¹⁰³, suggesting a somewhat conserved contribution
394 despite differences in cavity formation between mice and rats. Matrix deposition of type-1
395 collagen has also been described as a perivascular-fibroblast-derived scaffold for
396 neoangiogenesis⁶⁵. Therefore the role of collagen in fibrosis and angiogenesis in spinal cord
397 injury requires further study.

398

399 *Extracellular components are inhibitory to neural regeneration and plasticity*

400 In addition to an ongoing DAMP role for myelin debris, myelin-associated molecules confer
401 extrinsic inhibition to neurons. These include Nogo A, myelin-associated glycoprotein (MAG)
402 and oligodendrocyte myelin glycoprotein (OMgp). Nogo-A is a potent inhibitor to neural
403 plasticity and regeneration following spinal cord injury¹⁰⁴, preventing axons from overcoming
404 the spinal injury scar environment. Transmembrane receptor complexes are identified,
405 converging on the canonical RhoA/ROCK signalling pathway, resulting in destabilization of
406 the actin cytoskeleton and local arrest and collapse of growth cones¹⁰⁵. Furthermore, CSPGs
407 (Box 2) are upregulated by reactive glia following spinal cord injury both perilesionally and at
408 distal spinal segments^{98,106} and are associated with decreased plasticity and abortive
409 regeneration¹⁰⁷. Recent evidence suggests that scar-forming astrocytes express brevican,
410 and NG2, though not aggrecan¹⁰⁸. There is some evidence that core CSPG proteins are
411 inhibitory to neuronal growth¹⁰⁹ but CS-GAG chains are known to confer significant inhibition
412 following injury as their removal promotes anatomical and functional recovery following spinal
413 cord injury¹¹⁰. Membrane-bound receptors to CS-GAGs, reported to mediate inhibition, include
414 RPTP σ ^{111,112}, leukocyte common antigen-related phosphatase (LAR)¹¹³, NgR1 and NgR3¹¹⁴.
415 Signalling pathways implicated have convergence with those of Nogo and other myelin
416 inhibitors and include the Rho/ROCK pathway, activation of which is partly via PKC¹¹⁵ and
417 EGFR¹¹⁶ and coupled to Akt/GSK-3 activation¹¹⁷. CSPGs are also thought to inactivate neural
418 integrins¹¹⁸ and localise upregulated inhibitory guidance molecules such as semaphorin
419 3A¹¹⁹. Thus, the injured spinal cord contains molecules which restrict neurite outgrowth and
420 plasticity, and these are further upregulated and concentrated in the spinal injury scar and
421 represent therapeutic targets¹²⁰ (discussed below).

422

423 **Biomechanical properties of the scar**

424 Relatively little attention has been given to how the biomechanical environment of the injured
425 spinal cord affects repair¹²¹. Cells are highly mechanosensitive and changes in the elastic
426 properties of the environment alone can induce differentiation and migration¹²², and during
427 development mechanical gradients guide axon pathfinding¹²³. Astrocytes that are cultured on

428 less compliant, stiff substrates anatomically resemble reactive astrocytes, displaying
429 hypertrophy and elongation, with stiffness of CNS implants correlating with induction of
430 reactive astrocytosis¹²⁴. Integrin-mediated links between fibrillar type 1 collagen (known to be
431 stiffer in other tissues) and astrocyte reactivity are emerging⁵⁴, which may be influenced by
432 mechanotransduction. Atomic force microscopy has been used to characterize the
433 spatiotemporal elastic stiffness properties of spinal cord tissue over 1 to 3 weeks following
434 dorsal column crush lesion¹²⁵. At these early post-injury time points, tissue softened in areas
435 corresponding to scarring and ECM deposition. This was somewhat surprising because scar
436 tissue outside the CNS is typically stiffer than surrounding healthy tissue¹²⁶, and was attributed
437 to a lack of collagen-1 and loss of CNS myelin in these types of injuries (both of which scale
438 with tissue stiffness)^{127,128}, as well as the cellular composition of the scar (glial cells are softer
439 than peripheral scar myofibroblasts). It will be important to further characterize these
440 properties in more clinically relevant contusion-type injuries and in chronic injuries with
441 established scar tissue, particularly given the increasing evidence of a role for collagen-1 in
442 chronic contusive injuries^{54,63,129}.

443

444 **The spinal injury scar: the good, the bad, and the false-dichotomy**

445 **There has been some recent debate in the field on whether the scar is good or bad in terms**
446 **of recovery from injury^{108,130-132}. We propose that these two different viewpoints reflect different**
447 **interpretations of data which is, in fact, largely in agreement. Both historical and newer findings**
448 **support a long-established principle, that the spinal injury scar performs dual, and seemingly**
449 **opposing, roles; to protect tissue, and to inhibit repair.**

450

451 **As previously introduced, the classical description of the spinal injury scar is one which**
452 **considered the astrocyte-rich injury border alone, termed the glial scar.** Early observations of
453 dense glial reactivity at the site of CNS lesions led to the hypothesis that the **astrocytic** scar
454 inhibits axon regeneration, perhaps by forming an impenetrable barrier to axonal extension¹³³.
455 An inhibitory role for the scar and scar-associated molecules has been well documented ever
456 since **(as discussed above and^{26,106,107,134,135})**. However, it has also long been acknowledged
457 that the **astrocytic** scar has an important protective role **in enabling the separation of healthy**
458 **tissue from pathology following injury¹³⁶⁻¹³⁸**. Thus, for decades the dual notions that the scar
459 is associated with failed axonal re-connectivity **(inhibitory)** and also involved in a wound-
460 healing response **(protective)**, have existed.

461

462 **In addition, as discussed above, there is now increased appreciation of the multiple cell types,**
463 **beyond astrocytes, which contribute to spinal injury scarring, together with extracellular and**

464 non-neural components. This renders the term glial scar an insufficient descriptor. The
465 multifaceted nature of the scar should be considered when interpreting experimental
466 approaches which prevent scar formation. For example, a number of transgenic loss of
467 function experiments have been performed to specifically investigate the role of astrocytes
468 following spinal cord injury, including formation of the glia limitans and continued presence in
469 the chronic scar. Early evidence suggested that double knockout mice for the intermediate
470 filament proteins GFAP and vimentin (but not either protein alone) develop a less dense glial
471 scar, with greater haemorrhaging, fibrosis and presence of debris following lesion to the dorsal
472 funiculus¹³⁹. Similarly, conditional ablation of proliferating (scar-forming) reactive astrocytes
473 following injury increases edema, inflammation, oligodendrocyte death, tissue loss,
474 demyelination and functional deficits^{140,141}. Furthermore, deletion of SOCS3 or STAT3 in
475 astrocytes results in lesion expansion, cell death and exacerbated functional outcome^{58,92}.
476 Thus, a number of studies have provided evidence for the importance of reactive astrocytes
477 in preventing expansion of pathology into spared peri-lesional regions of the spinal cord. A
478 recent extension of these studies utilized these deletion strategies to specifically assess the
479 effects of reactive astrocytes on axonal regeneration¹⁰⁸. The study revealed that spontaneous
480 regrowth of damaged axons does not occur across a spinal crush injury following attenuation
481 or ablation of scar forming astrocytes, despite boosting the regenerative state of these axons
482 with a conditioning lesion and neurotrophin delivery¹⁰⁸. This study claimed to reveal a new
483 (and controversial) role of the astrocytic scar as being pro-regenerative. Our interpretation of
484 these findings, however, is that they are largely consistent with previous studies^{58,92,141}. A lack
485 of axon regeneration after ablating reactive scar astrocytes does not necessarily mean that
486 the glial scar aids axon regeneration. An alternative interpretation is that the regenerative
487 boost afforded by conditioning lesions and neurotrophin delivery is not sufficient to overcome
488 the prior-established lesion-exacerbating effects of preventing astrocytic scar formation.
489 Instead, injured axons are presented with neural and non-neural components of the spinal
490 injury scar, including NG2+ OPCs, inflammatory cells and CSPGs, all of which are known
491 blockers of regeneration¹⁴². Indeed, greater dieback of axons from the injury site was observed
492 in this study¹⁰⁸, in line with an advancing wall of inhibitory factors no longer contained in the
493 fibrotic lesion core. Thus, rather than overturning an old dogma, this study used elegant
494 genetic tools to demonstrate an important role for scar-forming astrocytes in tissue protection
495 following traumatic spinal cord injury, supporting previous observations^{58,92,141} and confirming
496 the early hypothesis postulated by Gopal D. Das: "If, by some means, glial scar formation
497 could be completely eliminated, most of the atrophying axons still might not show
498 regeneration, and the spinal cord would be continuously invaded by loose connective tissue
499 and other foreign materials and organisms while undergoing a protracted degeneration"¹³⁶.

500

501 Although astrocyte components of the spinal injury scar are well evidenced to be beneficial
502 initially, they have been suggested to be detrimental at a chronic stage^{32,143,144}. Worsened
503 functional outcome at 10 weeks post injury following diphtheria toxin-mediated ablation of
504 astrocytes at 5 weeks has been suggested to support a beneficial role of chronic scarring¹⁰⁸.
505 However, axon regeneration in this study may have occurred early, prior to scar maturation,
506 since axons were in a growth-stimulated state¹³¹. It is possible that astrocyte ablation at both
507 early and chronic stages is detrimental, however further studies will be needed to establish
508 this. A recent proteomic analyses of subacute (2 week) and chronic (8 week) spinal injury scar
509 tissue revealed differential features in the different post-injury phases, with abundant
510 expression of growth factors observed subacutely but not chronically, where the signature
511 instead showed high expression of growth inhibitory ECM molecules including collagens and
512 CSPGs¹⁴⁵. This study also demonstrated increased axonal regeneration across a complete
513 spinal transection injury after surgical removal of chronic scar tissue. Taken together, this data
514 supports a beneficial role for early scarring and an inhibitory role for chronic scarring. It will be
515 important to further characterise the molecular profile of the spinal injury scar at precise time
516 points after injury, both for the cell types involved in scarring (e.g. Box 1) as well as features
517 of the environment.

518

519 Thus, the dual nature of the spinal injury scar has long been known, yet continues to be
520 reviewed, revisited and reinterpreted^{19,64,92,108,132,143,144,146}. Rather than focus on good versus
521 bad, perhaps efforts would be best directed at understanding and targeting specific aspects
522 of the scar to aid recovery. For example, it may be beneficial to target components of the scar
523 which are non-permissive to regeneration or plasticity rather than removal of the astrocytes
524 themselves, even at chronic time-points. Targeting diverse cell types and phenotypes, as well
525 as extracellular and non-neural components should also be considered, as well as the timings
526 of such interventions. These approaches will be discussed in the following section.

527

528

529 **Therapeutic strategies**

530 Current experimental approaches for targeting the spinal injury scar attempt to either reduce
531 scar formation, or to block inhibitory molecules associated with the scar, using a variety of
532 surgical, pharmacological and genetic approaches. Some of these show promise for
533 application in the clinic.

534

535 *Attenuating scar formation*

536 Although preventing formation of the astrocytic component of the spinal injury scar impacts
537 negatively on wound-healing (discussed above), as the mechanistic understanding of spinal

538 **injury scar** pathology increases, a number of studies in preclinical rodent models have targeted
539 mesenchymal or fibrotic-derived components in a bid to limit amplification of tissue damage.
540 On a gross tissue scale, if the dura is breached, dural apposition and/or patching with another
541 soft tissue material (duraplasty) is suggested to limit fibrotic and connective tissue deposition
542 from meningeal-derived fibroblasts¹⁴⁷. Decompressive durotomy followed by dural allograft
543 has been shown to reduce scar formation and lesion volume, but if the dura is not replaced,
544 lesion volume increases dramatically¹⁴⁷ and **indeed expansion duroplasty is performed**
545 **alongside decompressive durotomy in clinical evaluations**¹⁴⁸.

546

547 The **fibrotic components of the scar** can also be targeted **pharmacologically**. Systemic
548 administration of **the microtubule stabilizing antimetabolic agents taxol or Epothilone B** leads to
549 reduced migration of scar-forming fibroblasts and suppression of extensive scar formation,
550 **enabling axon regeneration and functional recovery**¹⁴⁹⁻⁵¹. This highlights the potential for
551 **repurposing of epothilones and taxanes that are already used in cancer treatment**¹⁵⁰. Inhibiting
552 **collagen synthesis using the iron chelators BPY-DCA (which inhibits prolyl 4-hydroxylase, a**
553 **key enzyme of collagen IV synthesis) and cyclic adenosine monophosphate (cAMP, which**
554 **inhibits meningeal fibroblast proliferation)**^{151,152} **reduces fibrotic scarring and promotes**
555 **neuroprotection and long-distance axon regeneration**¹⁵¹. Treatment with clinically approved
556 ion chelator deferoxamine and inhibition of lysyl oxidase, another key collagen biosynthetic
557 enzyme, also improves outcome after partial spinal transection injuries in rodents^{153,154}.

558

559 **The gene expression profile or phenotype of astrocytes may also represent potential**
560 **therapeutic targets with modulating effects on scar formation. For example, selective inhibition**
561 **of NF κ B signalling in astrocytes reduces inflammation and is pro-reparatory in mice**
562 **expressing a dominant negative K β under the GFAP promoter**⁹³. However, this is difficult to
563 **target therapeutically. If current studies of astrocyte phenotype in the mouse brain (Box 1)**
564 **translate experimentally to the injured spinal cord, newly identified gene/signalling targets and**
565 **matrix targets (below) could be manipulated to depress propagating scar pathology. *In vitro*,**
566 **application of human recombinant TGF β 3 (but not the removal of mediators IL1 α , TNF and**
567 **C1q) was able to rapidly reverse transformation from an A1 phenotype to an unreactive**
568 **status**⁹⁴. Following optic nerve crush, this phenotypic conversion was demonstrated *in vivo* by
569 **delivery of antibodies to IL1 α , TNF and C1q**⁹⁴. Manipulation of TGF β 3 was not reported *in*
570 ***vivo*, however multiple studies have targeted TGF β 1&2 following spinal cord injury**¹⁵⁵ to reduce
571 **spinal injury scar formation, so it would be interesting to know whether this is a TGF β 3-specific**
572 **effect. Indeed TGF β 1 and 2 are known to exert opposing effects to TGF β 3 on wound healing**

573 outside of the CNS, and human recombinant TGF β 3 has been utilized in clinical trials to
574 promote dermal wound healing and scar reduction¹⁵⁶.

575

576

577 *Targeting the extracellular matrix*

578 Following spinal cord injury, the extracellular environment contains molecules which interact
579 directly with neurons and other cell types (discussed above). Some of these are thought to
580 augment pathology and degree of spinal injury scarring, while some directly inhibit the ability
581 of neurons to overcome a scar environment and generate novel connectivity. Both are
582 potential therapeutic targets.

583

584 Periostin is a secreted ECM protein which has recently been implicated in contributing to scar
585 formation, via propagating fibrosis and inflammatory signalling^{50,70}. Daily intraperitoneal
586 injections with a mouse monoclonal antibody against periostin from 4 days to 2 weeks after
587 injury was shown to reduce the extent of tissue pathology and scarring, which led to functional
588 improvements in sensorimotor tasks following contusion injury in mice⁷⁰. Generation of
589 recombinant anti-periostin will allow this promising strategy to be further tested. Similarly, a
590 recent study found that pathology could be attenuated within the first 2 weeks following spinal
591 cord injury in mice treated with an N-cadherin neutralizing antibody, which blocked an integrin
592 and N-cadherin dependent interaction between extracellular type-1 collagen and astrocytes
593 and significantly attenuated astrocytic scar formation⁵⁴. In this study the rapid behavioural
594 recovery observed supports a neuroprotective role for neutralising n-cadherin, though this was
595 not assessed directly. As above, these studies suggest that early fibrosis is an important
596 therapeutic target for improving outcome after spinal cord injury. Furthermore, if A1/A2
597 polarisation factors⁹⁴ are conserved following spinal cord injury, a known inhibitor of C1q is
598 chondroitin sulfate A¹⁵⁷, the mono CS-4 sulfated GAG, which raises interesting questions
599 regarding additional roles of ECM proteoglycans and their sulfation epitopes following injury.
600 Thus, matrix properties are a valuable means to tap into the plasticity of cell responses.

601

602 CSPGs (see Box 2) are known inhibitors of neuronal plasticity, present throughout the CNS
603 ECM and highly concentrated in the spinal injury scar (discussed above). A number of
604 experimental studies have reported functional improvement following spinal cord injury by
605 genetic removal of enzymes critical for CS-GAG biosynthesis. This includes deoxyribozyme-
606 mediated knockdown of xylosyltransferase-1 mRNA, the enzyme which catalyses GAG
607 addition to the CSPG core protein¹⁵⁸, conditional sox9 ablation¹⁵⁹ and knockout of N-
608 acetylgalactosaminyltransferase-1, the enzyme which catalyses the addition of the first
609 GalNAc residue onto the tetrasaccharide link between the core PG and GAG¹⁶⁰. Reports of

610 therapeutically-applicable pharmacological approaches which recapitulate these effects are
611 currently lacking.

612

613 Enzymatic strategies targeting CSPGs are a promising approach for spinal cord repair, due to
614 their ability to render the ECM more permissive to neuronal plasticity and connectivity.

615 Removal of CS-GAGs by the chondroitinase ABC (ChABC) enzyme has been widely
616 demonstrated to have beneficial effects in enhancing axonal regeneration and neuroplasticity
617 and promoting functional recovery following experimental spinal cord injury^{110,161,162-164}. This
618 effect has been replicated across multiple laboratories and in different species¹⁶⁵, including
619 mouse, rat, cat, and recently in primates¹⁶⁶ and in a canine clinical model¹⁶⁷. Furthermore, its
620 use as an adjunct therapy can augment the benefits of other experimental therapeutics¹⁶⁸⁻¹⁷⁰.

621 A gene therapy method of enzyme delivery, where host cells are themselves transduced to
622 express the ChABC gene leads to extensive CS-GAG digestion, which results in reduced
623 pathology and improved functional recovery following contusion injury to the thoracic¹⁷¹ and
624 cervical^{172,173} spinal cord. Furthermore, widespread CSPG modulation achieved by viral
625 delivery of ChABC promotes conversion of macrophages towards a pro-resolving M2
626 polarization state¹⁷¹ and drives an anti-inflammatory IL-10-mediated response¹⁷⁴, which is
627 likely to underlie reduced pathology. Thus, ChABC is a promising means to promote resolution
628 of pathology as well as overcoming the inhibitory environment of the spinal injury scar. A
629 recent study utilised a novel gene switch to enable controlled delivery of the ChABC gene and
630 revealed that long term ChABC gene expression was required to elicit recovery of skilled reach
631 and grasp functions, with recovery attributed to plasticity of descending systems¹⁷³. Whether
632 this viral ChABC approach will also have benefit when applied chronically is not yet
633 established. However, recent work has demonstrated that a single injection of ChABC enzyme
634 in the phrenic motor pool 1.5 years after unilateral cervical spinal cord injury was able to elicit
635 rapid and robust recovery of respiratory function, restoring the ventilatory response to the
636 paralysed hemidiaphragm¹⁷⁵. Furthermore, chronic application of ChABC prior to
637 transplantation of induced pluripotent stem cell-derived neural stem cells 7 weeks after a
638 spinal compression injury led to reduced chronic-injury scarring, increased graft survival and
639 improved limb function¹⁷⁶. These studies highlight the potential for ChABC to unmask latent
640 neuroplasticity and produce a microenvironment conducive to repair even within the chronic
641 spinal injury scar.

642

643 Another enzymatic strategy that has recently been exploited for reducing CSPG inhibition is
644 the mammalian enzyme Arylsulfatase B (ARSB, N-acetylgalactosamine-4-sulfatase), which
645 removes C4S moieties specifically from CS-GAGs. In addition to being utilized in enzyme-
646 replacement therapy for human mucopolysaccharidosis VI, ARSB administration has now

647 been shown in one study to promote increased axonal sprouting and functional locomotor
648 recovery following compression spinal cord injury in the mouse¹⁷⁷. ARSB perhaps represents
649 a more attractive, and more readily translatable, therapeutic prospect than a bacterial enzyme
650 such as ChABC and certainly warrants further investigation, particularly given recent findings
651 of eliciting enhanced axon growth in the injured optic nerve¹⁷⁸. However, whether ARSB could
652 elicit robust modulatory effects within the spinal injury scar microenvironment, comparable to
653 ChABC, remains to be determined. Given the specificity of ARSB for 4S motifs, it may not be
654 capable of the multi-modulatory effects that have been demonstrated for ChABC which include
655 immune modulation, neuroprotection and neuroplasticity^{165,179,180}.

656
657 Targeted modulation of CSPG receptor signalling via manipulation of the receptor PTP σ , has
658 also proved to be a promising therapeutic prospect. The activity of the intracellular
659 phosphatase domains of PTP σ are regulated via a conserved “wedge” structure which can
660 occlude the catalytic domain, thus reducing phosphorylation activity and ability to signal
661 downstream. Use of a membrane-permeable peptide mimetic of this wedge reduces PTP σ
662 signalling following activation by ligands such as CSPGs. Systemic delivery of this peptide
663 has been shown to enable recovery of locomotor and bladder function in rats following spinal
664 contusion injury¹⁸¹ and the non-invasive nature of this approach means it is a realistic
665 candidate for rapid translation. Thus, approaches to overcome the inhibitory actions of CSPGs
666 show collective promise in enabling beneficial alterations to the ECM associated with the
667 spinal injury scar with positive effects in eliciting some functional repair.

668
669 *Future directions for therapy*

670 The majority of approaches aimed at manipulating the spinal injury scar for therapeutic benefit
671 have focused on modifying scar-associated ECM and targeting the synthesis, production and
672 signalling of CSPGs. With the identification of cell subtypes that have opposing actions on
673 tissue pathology, such as A1 neurotoxic vs A2 reparatory astrocytes⁹⁴, there may be further
674 opportunity to modulate astrocyte function or phenotype following spinal cord injury. These
675 approaches will likely evolve as new markers are identified for delineating reactive astrocytes
676 and microglia in different phenotypic states¹⁸², with increasing availability of astrocyte and
677 microglia cell-specific sequencing data^{54,79,108} and with powerful emerging tissue sequencing
678 technology¹⁸³. Alternative approaches which may indirectly modulate astrocyte phenotype are
679 also emerging, such as grafting specific stem cell populations which can influence host tissue
680 cellular responses and drive astrocyte transformation to a permissive phenotype¹³².
681 Additionally, with increased appreciation for the role of ECM molecules in affecting pathology
682 and plasticity of cellular responses, novel targets may be identified with new matrix biology

683 technology¹⁸⁴. Conversely, the study of scarring mechanisms and ECM components in
684 organisms that are capable of CNS regeneration may lead to the identification of pro-repair
685 targets. For example, differential regulation of collagen XII within the scar matrix is one factor
686 contributing to the pro-regenerative phenotype in zebrafish, controlled by Wnt/B catenin
687 signalling¹⁸⁵. Whether Wnt/B-catenin-mediated collagen XII production can be harnessed to
688 render the mammalian spinal injury scar more permissive is not yet known. Finally,
689 consideration should be given to scar biomechanics^{121,125} when designing therapies. It may be
690 important to understand how pharmacological manipulations affect mechanobiology and
691 further provide appropriate mechanical signals to optimize repair.

692

693 **Conclusion**

694 The spinal injury scar is multifaceted. It contains more than just a reactive glial component
695 and should be considered as a whole, since there is a complex interplay between multiple
696 different cell types (glial cells, mesenchymal-derived cells, immune cells), their intracellular
697 and signalling changes, and the extracellular environment. These processes modulate and
698 feedback on each other. Altering the environment, for example using CSPG modulation with
699 chondroitinase, can increase neuronal regeneration-associated gene expression and
700 transcriptional changes^{110,174}. Conversely, altering intracellular mechanisms can alter the
701 inhibitory environment (for example microtubule stabilization with taxol or epothilone B leads
702 to reduced fibrotic scarring¹⁴⁹). Similarly, astrocyte-immune cell interactions are bidirectional,
703 where an increasingly proinflammatory environment induces extensive astrogliosis⁵³ and in
704 turn, activated astrocytes release pro-inflammatory cytokines, chemokines and CSPGs, which
705 can influence the magnitude of the inflammatory response²². The spinal injury scar has both
706 beneficial properties (being essential for preventing spread of cellular damage) and
707 detrimental properties (limiting new growth and tissue repair). This may be attributable to
708 opposing phenotypes of reactive glial cells that form the scar border, given recent evidence in
709 other CNS pathologies⁹⁴. However, also important to note are the opposing roles of the scar
710 matrix which contains beneficial molecules, required for formation of the glia limitans (which if
711 not formed properly, can increase damage and worsen outcome) as well as molecules that
712 are potent inhibitors of growth and neuroplasticity, such as CSPGs. Therapeutic strategies
713 need to target detrimental aspects while preserving the beneficial properties of the spinal injury
714 scar. Increased mechanistic understanding of the biological processes that propagate the non-
715 resolving scar pathology is providing new therapeutic targets which may bring us closer to
716 improving functional outcome following traumatic spinal cord injury.

717

718

719 **Box 1: Phenotypic diversity and plasticity of astrocytes: emerging evidence from**
720 **brain and spinal cord injury**

721 There is increasing data available on the cellular profile and phenotypic diversity of astrocytes
722 after injury. Phenotypic nomenclature comparable to that adopted in characterization of
723 macrophages/microglia has been used for the transcriptional profiling of cultured astrocytes
724 isolated from the CNS under different injury conditions. Ischemic injury in the brain (modelling
725 stroke) leads to a trophic A2 polarization state. By contrast, activated microglia from models
726 of neurodegenerative and neuroinflammatory diseases and traumatic optic nerve crush injury
727 release factors such as IL1 α , TNF and complement component subunit 1q (C1q) which induce
728 a neurotoxic state in A1 astrocytes^{55,94}. In the optic nerve, there is evidence that axotomy-
729 induced A1 astrocytes, in turn, kill axotomized neurons. In these studies, microglial-derived
730 mediators were necessary and sufficient to induce an A1 phenotype. Whether this occurs
731 following spinal cord injury is, as yet, unreported.

732
733 Genetic profiling of reactive astrocytes and scar-forming astrocytes following spinal cord injury
734 (isolated by laser-capture microdissection at 7 and 14 days post injury, respectively) has
735 recently been reported. Reactive astrocytes were associated with selective upregulation of
736 *Nes*, *Ctnnb1*, *Axin2*, *Plaur*, *Mmp2*, and *Mmp13* whereas scar-forming astrocytes selectively
737 upregulated *Cdh2*, *Sox9*, *Xylt1*, *Chst11*, *Csgalnact1*, *Acan*, *Pcan* and *Slit2*⁵⁴. Furthermore,
738 using these genes as population markers, FACs isolated nestin-GFP+ reactive astrocytes
739 were found to convert to a naïve phenotype following transplantation into uninjured tissue but
740 become scar-forming when transplanted into injured tissue, an effect thought to be mediated
741 by a N-cadherin-dependent interaction with type 1 collagen⁵⁴. Thus, astrocytes are able to
742 display phenotypic plasticity, and tissue environment is a crucial influence over cellular
743 behaviour.

744
745 The GFAP-RiboTag mouse can be used to perform high-throughput RNA sequencing on
746 astrocytes following injury and probe the effect of particular genes in the astrocyte response¹⁰⁸.
747 Two weeks after a spinal crush injury RNA-seq revealed differential expression of over 6000
748 genes in astrocytes, changes described as congruent with prior transcriptomic analysis
749 following ischemic stroke lesion^{55,108}. However, whether the astrocytes sampled here
750 represent A2-like trophic astrocytes has not been ascertained.

751
752 There are some discrepancies between gene expression findings using these different
753 methodologies. For example, of the 8 genes associated with scar-forming astrocytes isolated
754 using laser capture microdissection⁵⁴, the RNA-seq dataset only supports increased
755 expression in one of these (*Xylt1*)¹⁰⁸, whereas 4 of the 6 genes ascribed to reactive

756 astrocytes⁵⁴, are increased (*Nes*, *Axin2*, *Plaur*, *Mmp2*)¹⁰⁸. Spatial differences in sample
757 selection may contribute to these disparities. Techniques such as 3D intact-tissue RNA
758 sequencing¹⁸³ may overcome this problem in the future. Indeed, further characterization of
759 spatio-temporal phenotypic diversity and plasticity would aid research into how astrocyte
760 phenotype and scar progression may be modified by changes to matrix components or
761 perturbation of the immune response. Such methods, alongside new purification techniques
762 for *in vitro* analysis, will likely provide increasingly nuanced understanding of the relationship
763 between astrocytes, microglia, non-resident immune cells and the tissue environment^{79,186}.

764

765

766 **Box 2: Structure of chondroitin sulfate proteoglycans (CSPGs)**

767 CSPGs are proteoglycans (PGs) consisting of a core protein with at least one covalently
768 attached chondroitin sulfate glycosaminoglycan (CS-GAG) side chain. CSPG subtypes most
769 commonly studied with respect to the inhibitory CNS environment include lecticans (aggrecan,
770 versican, neurocan and brevican), the transmembrane protein NG2, phosphacan
771 (transmembrane or soluble) and the small leucine-rich proteoglycans decorin and biglycan.
772 There are also multiple less-well studied CSPGs revealed by proteomics analysis of scar
773 matrix⁵⁰. Lecticans are the most abundant CSPGs in the spinal injury scar and also feature
774 globular domains: the G1 N-terminal domain and G3 C-terminal domains are important in their
775 interaction via link-protein with hyaluronan (the backbone glycoprotein of the CNS matrix) and
776 also tenascin, thus they are involved in matrix crosslinking. Core PGs undergo post-
777 translational modification in the endoplasmic reticulum and golgi, catalysed by a number of
778 enzymes. At particular serine residues a tetrasaccharide linking region is formed by sequential
779 addition of xylose by xylosyl transferase, two galactose molecules by β 1,4-
780 Galactosyltransferase-I then β 1,3-Galactosyltransferase-II and a one GlcA residue via β 1,3-
781 glucuronyltransferase I to form the linker GlcA β 1-3 Gal β 1-3 Gal β 1-4 Xyl β 1-O-Ser. The
782 following addition of GalNac by a GalNac transferase I is crucial to initiate synthesis of the
783 chondroitin sulfate backbone. If, at this point, N-acetylglucosamine (GlcNac) is added rather
784 than GalNac, synthesis of the heparan sulfate backbone is initiated.¹⁸⁷ CS-GAG chain
785 polymerisation is the process by which alternating residues of GalNac and GlcA are then
786 added to the proteoglycan linker region by the alternating activity of a GlcA transferase II and
787 a GalNac transferase II. There are six actual enzymes identified which confer this
788 glycosyltransferase activity. Alongside CS-GalNac transferase I and II, combinations of
789 enzyme complexes of chondroitin synthase 1,2 and 3 and chondroitin polymerising factor
790 (ChPF) mediate GAG polymerization.

791

792

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798

799 **Competing interests**

800 The authors declare no competing interests.

801

802 **Author contributions**

803 EJB and ERB wrote the Review; ERB prepared the figures, with input from EJB.

804

805 **Figure Legends**

806 **Figure 1.** *Cellular and extracellular composition of the spinal injury scar.*

807 Traumatic spinal cord injury triggers a complex cascade of events that culminate in the spinal
808 injury scar which consists of multiple cell types as well as extracellular and non-neural
809 components. **a)** in the acute post-injury phase (0-72 hours), cell death and damage lead to
810 release of a number of cellular and blood-derived DAMPs (damage associated molecular
811 patterns). These are powerful activating and inflammatory stimuli for stromal cells, astrocytes,
812 NG2+OPCs and microglia. Fibroblast-like cells proliferate from perivascular origin. Activated
813 cells increase deposition of extracellular matrix molecules such as chondroitin sulfate
814 proteoglycans (CSPGs) and stromal-derived matrix. Circulating immune-responders
815 (neutrophils, monocytes) are recruited, their relative expression of cytokines, chemokines and
816 matrix metalloproteinases becomes shaped by the early injury environment, and a mixed
817 immune cell phenotype (M1, pro-inflammatory; M2, pro-resolving) is initially adopted. This
818 becomes increasingly proinflammatory. **b)** in the chronic spinal injury scar, monocyte-derived
819 macrophages/microglia adopt a predominantly M1 phenotype. Rather than entering a phase
820 of resolution, responding innate immune cells present DAMPs to circulating adaptive immune
821 cells and pathology spreads. Reactive astrocytes hypertrophy, upregulate expression of
822 intermediate-filament associated proteins and secrete matrix CSPGs. Fibroblast-like cells
823 contribute to fibrotic tissue remodelling and deposition of stromal-derived matrix. Innate
824 immune cells become unable to process cellular and matrix debris effectively and become
825 synonymous with lipid-rich foam cells. Scar-forming reactive astrocytes organise into a barrier-
826 like structure which separates spared tissue from a central region of inflammation and fibrosis
827 where wound-healing fails to undergo resolution. In most mammalian species a chronic cystic
828 cavity develops. Wallerian degeneration of injured axonal projections contributes to continued
829 extracellular deposition of axonal and myelin debris, which is ineffectively processed by

830 immune cells, and along with CSPGs, acts to inhibit neuronal regeneration and neuroplasticity
831 long-term.

832

833 **Figure 2.** *From injury to scar: time course of progressive scar pathology showing interlinked*
834 *relationships between different components of the spinal injury scar.*

835 Following traumatic spinal cord injury, acute cell death and damage triggers release of cell-
836 derived and blood-derived DAMPs, ATP release, dysregulated ionic homeostasis oxidative
837 stress and excitotoxicity, which represent potent stimuli for triggering glial cell activation,
838 stromal cell proliferation, deposition of extracellular matrix (ECM), and recruitment of
839 circulating innate immune cells. Within a few days following injury, monocyte-derived
840 macrophage/microglia adopt a predominantly “M1” phenotype which do not favour resolution
841 and tissue remodelling becomes fibrotic. Proinflammatory innate responders also present
842 DAMP-derived antigens (such as MBP) to T and B-cells. B cells, in turn, may present antigens
843 to T-cells, triggering their expansion. During this time, reactive astrocytes proliferate,
844 hypertrophy and overlap in order to isolate this zone of nonresolving pathology from spared
845 tissue. They also secrete matrix CSPGs, which are known to downregulate neuronal plasticity.
846 Wallerian degeneration of degenerating axonal tracts contributes to continued deposition of
847 axonal and myelin debris, which is ineffectively processed by immune cells and leads to the
848 deposition of myelin-associated molecules (MAG, Nogo, OMgp) which are known inhibitors of
849 neuronal regrowth. Ongoing Wallerian degeneration at later post-injury stages further triggers
850 gliosis and neuroinflammation. Dashed grey arrows show cross talk between different
851 components of the spinal injury scar, which is usually bidirectional. For example, CSPGs
852 released by reactive astrocytes are thought to activate receptors on macrophages/microglia
853 to induce a proinflammatory phenotype and in turn increasing inflammation induces further
854 astrocytic reactivity and CSPG deposition. Fibroblast-like cells also synthesise type 1
855 collagen, implicated in the induction of astrogliosis and further deposition of matrix molecules.
856 Cross talk between the innate and adaptive immune response also propagates inflammatory
857 pathology and further influences glial activation and CSPG production. The dynamic
858 interactions between inflammation, dramatic tissue and ECM remodelling and reactive cellular
859 and extracellular changes drive the progressive, propagating pathology that culminates in the
860 spinal injury scar.

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