ACS Biomaterials SCIENCE & ENGINEERING
Cite This: ACS Biomater. Sci. Eng. 2017, 3, 2744-2760
Review
pubs.acs.org/journal/abseba

Techniques for Multiscale Neuronal Regulation via Therapeutic Materials and Drug Design

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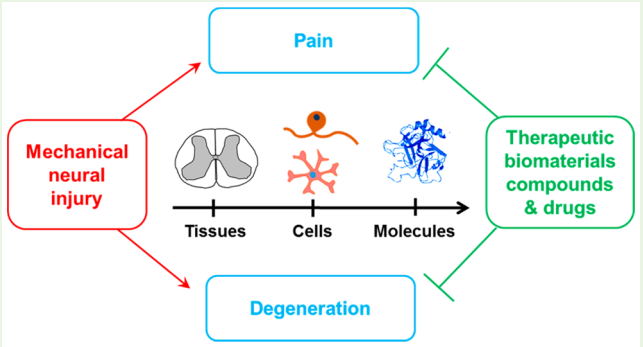
ABSTRACT: Neurotrauma is a common source for a host of neurological disorders, including chronic pain. Pathological changes underlying neural injury and pain are complex due to the multiscale spatiotemporal nature of the nervous system and its response to insults. Understanding the combined influence of tissue mechanics, neuronal and glial activation, and molecular processes on the development and maintenance of pain has recently gained attention. The growing knowledge about nociceptive mechanisms has inspired the design of novel therapeutic materials and compounds for neuronal regulation. Primary mechanical insults and secondary inflammatory responses can induce morphological changes, electrophysiological abnormalities, and altered neurotransmitter release associated with neuronal dysfunction, degeneration, and/or death in both central and peripheral nervous systems. Such responses in afferent and spinal dorsal horn neurons directly and indirectly potentiate pain. Using separate radiculopathy and joint pain models, the mechanical, nociceptive, and inflammatory aspects of pain are reviewed. In that context, biomaterials and compounds with material advantages, neuroprotective benefits, or anti-inflammatory effects to mitigate pain are identified. Several promising techniques to promote neuronal survival and axonal regeneration after injury, including bioactive scaffolds, blocking growth-inhibitory molecules, and active drug delivery, are highlighted. Similar biomaterials-based strategies and molecular intervention have shown promise in attenuating various types of pain. Advancing these and other approaches will help advance and deepen the mechanistic understanding underlying trauma-induced pain across different length scales.



KEYWORDS: *neurotrauma, pain, mechanical injury, radiculopathy, joint, regeneration, therapeutic biomaterials, active targeting, multiscale*

Traumatic insults to the central nervous system (CNS) or the peripheral nervous system (PNS) can induce a host of injury responses across different spatial scales. Changes, which include alterations in the affected tissues, the function of the involved cells, and a variety of molecular pathways, are not limited to the site of the injury. Yet, such responses can also become widespread depending on the biomechanical and biochemical properties of the injured tissue and lead to impaired function and health.[1−4] Tissues in the nervous system reside in different mechanical environments and have heterogeneous material properties due to varied composition and structure, resulting in distinct mechanical tolerances and vulnerabilities to trauma.[1,5] Responses of neural tissues to mechanical loading are highly dependent on the rate and magnitude of the applied force and/or deformation.[6−9] High-rate loading limits the time for these viscoelastic tissues to adapt and relax, and large loads can exacerbate structural impairment and further increase the injury severity.[1,5] Stresses that exceed the physiologic range can disturb tissue homeostasis and produce tissue dysfunction, including altered cellular composition and disorganization of the extracellular matrix (ECM).[10−13] In the injury domain, extreme loading can produce a tissue rupture or lesion and even tissue death.[5,10] Physical distortion and structural failure of nervous tissue are not the only causes of deleterious outcomes after traumatic neural tissue insults. Secondary complications, including cellular infiltration, neuronal and glial activation, and regulation of molecular signals, can amplify the primary insult and exacerbate the consequences of neural injury.[1,10,13−15] Linking macro-

Special Issue: Multiscale Biological Materials and Systems: Integration of Experiment, Modeling, and Theory

Received: January 5, 2017
Accepted: February 23, 2017
Published: February 23, 2017

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

scopic tissue responses to microscopic modifications can be confounded by different structural and functional thresholds and varied time courses across spatiotemporal scales. The fact that the nervous system involves multiple hierarchical levels requires investigating the cellular and molecular mechanisms of traumatic neural injury and an equally important need for designing multiscale strategies for treating it when injured (Figure 1).

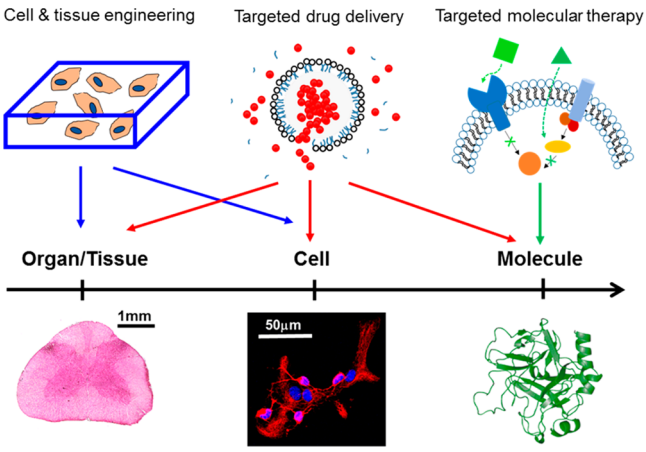


Figure 1. Multiscale treatment strategies for neural repair. Biomaterials-based therapies for neural regulation target the nervous system at different levels across the organ-to-tissue-to-cell-to-molecule levels. Tissue engineering approaches, such as bioactive scaffolds, can be used to replace damaged tissues and cells, whereas molecular and gene therapies promote repair by modulating neuronal viability and signaling. Novel drug delivery routes, such as active targeting with nanoparticles, may enhance the treatment integrity across the length scale.

Traditional multiscale studies in the field of biomaterials connect molecular-scale chemistry and cellular responses to mesoscale and macroscale material properties. In the context of neuronal regulation and treatments, multiscale mechanisms and therapies refer to hierarchical paradigms involving molecules, cells, and tissues for studying and treating neural injury and pain (Figure 1). This review focuses on presenting the relevant injury mechanisms for pain from neural trauma at multiple length scales (multiscale) and, in so doing, highlights potential treatment strategies that individually and/or simultaneously intervene different macroscopic and microscopic components in the nervous system to control pain. We first provide a brief overview of the general cellular responses and regulatory mechanisms following neurotrauma. With that context, we next emphasize neuronal regulation of pain after two main types mechanical injury: direct neural insults and loading to innervated tissues. We discuss how multiscale biochemical and biomechanical techniques are employed to understand the pathophysiology of pain and to reveal potential therapeutic targets using those examples. On the basis of those pathological discoveries, biomaterials-based strategies that target the damaged tissue, neurons and glia, and molecular pathways have been developed for neural repair and functional recovery. Such treatment approaches have shown great promise in attenuating pain. Three examples are reviewed to demonstrate how therapeutic materials and compounds are used to study and treat pain across different length scales.

NEURAL REGULATION AFTER TRAUMATIC INJURY

Traumatic neural injury induces progressive neurodegeneration, leading to impairment and/or loss of cognitive, sensory, and motor function depending on the site of injury.[1,14,16] Neurodegeneration and neural dysfunction are mediated by changes in neuronal structure, electrophysiological properties, and neurotransmitter release.[15,17,18] As a result of excessive neural loading, neurons can undergo membrane leakage, mechanoporation of the cell membrane and axolemma, and disruption of cytoskeletal components.[14,15] Interrupted axonal transport and abnormal axonal morphology, like focal swelling and retraction bulbs, can occur within a few hours after injury.[19,20] Mechanical disruption of microtubules is a primary cause of axonal abnormalities in the early post-traumatic period;[19,21] stabilizing microtubules after spinal cord injury (SCI) can reduce scarring and promote neural regeneration.[22] Damaged axons also undergo Wallerian degeneration weeks to months after traumatic injury.[23] In addition to morphologic modifications, a shift in electrophysiological responses also occurs after neural injury.[4,7,18] Injured neurons can exhibit abnormal firing patterns and sustained ectopic activity, such as a shift in their latency, persistent after-discharge and increased firing rate when stimulated.[8,18,24] The electrical signals in adjacent intact axons can also increase, and there are immediate changes in neural network activity reported after traumatic injury.[4,24−26] Membrane depolarization is typically accompanied by increased gene expression and release of neurotransmitters, including glutamate and other excitatory amino acids.[15,27,28] Changes in both neuronal excitability and the interruption of ionic equilibrium that is induced by disruption of the neuronal membrane can modify the influx of ions, especially Ca^{2+}, triggering a cascade of subcellular events that lead to cell death.[15,27,28]

Secondary events following a primary neural insult elicit robust inflammatory responses that play dual roles in neural tissue repair and regrowth.[15,29,30] Traumatic injury to the CNS can break down the blood−brain barrier or blood−spinal cord barrier (BSCB) and active glial cells, both enabling the infiltration of immune cells to the injured tissue and release of inflammatory mediators that modify the biochemical environment surrounding the neurons.[31,32] Unfortunately, neurons in the CNS have very limited regenerative ability, and the role of inflammation in CNS repair after trauma is controversial and inconclusive.[29] For instance, SCI induces a robust inflammatory response that has both beneficial and detrimental effects. Following the breakdown of the BSCB after spinal cord trauma, leukocyte adhesion molecules on the surface of the endothelial cells are rapidly increased. This increase in adhesion molecules leads to recruitment of peripheral leukocytes to the injury site and its surrounding tissue, accompanied by synthesis of pro- and anti-inflammatory cytokines and chemokines.[30,33] Immune cells, like macrophages, microglia, and astrocytes also become activated and regulate the neural system environment by removing cellular and ECM debris and secreting molecular mediators that regulate either degenerative or regenerative processes.[30,34] Yet, axon regeneration across the injury site following SCI is typically prevented by glial scarring.[29,35] In contrast to axons in the CNS, those in the PNS not only have greater intrinsic capacity to upregulate regeneration-associated genes but also can benefit from inflammation and reside in a growth supportive microenvironment that is not present in the

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

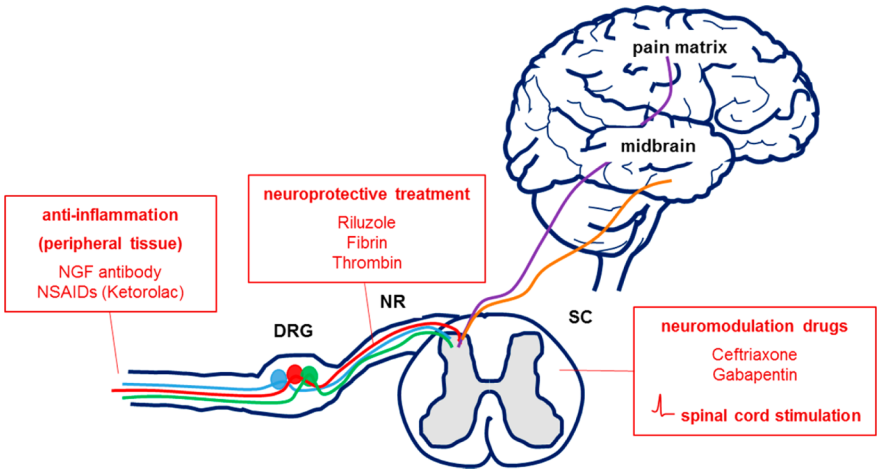


Figure 2. Schematic illustration of the pain pathways and exemplar treatment strategies for neural injuries of different pathologic etiologies. Afferent neurons that innervate peripheral tissues have their soma in the dorsal root ganglion (DRG). Their axons form the dorsal nerve root (NR), extending from the DRG and synapsing in the spinal cord (SC). Primary afferents transmit nociceptive information to the brain through higher order pathways. Pain can be attenuated by therapeutic interventions targeting any and/or all of those anatomical structures. Anti-inflammatory treatment given intra-articularly can attenuate joint pain. Neuroprotective compounds and drugs are effective in alleviating radicular pain via direct application to the neural tissue. Once pain is centralized in the SC it can also be mitigated using neuromodulation techniques.

CNS.[29,35,36] Pro- and anti-inflammatory responses regulate the PNS microenvironment involving the injured distal nerve stump and glial and immune cells to aid in axon regeneration.[29,37,38] The disintegration of the axoplasm in the PNS that occurs within a few days following neurotrauma attracts macrophages to the site of injury.[39−42] Those infiltrating macrophages not only remove degenerated axons and phagocytize damaged myelin but also release factors that mediate the regrowth of axons.[38,43] The cytokine interleukin-1β (IL-1β) is an exemplar molecule that promotes regeneration by modulating the secretion of the neurotrophic factor nerve growth factor (NGF) from Schwann cells and fibroblasts.[44] The beneficial effects of NGF on peripheral nerve regeneration are well-defined.[45,46] Neurotrophin-guided recovery is accelerated by inflammation since circulating macrophages are recruited to the injured peripheral tissue.[29,38] As part of the inflammatory cascades initiated after trauma, Schwann cells proliferate and produce inflammatory cytokines that recruit blood-borne macrophages to the area and contribute to phagocytosing debris.[29,35] They also migrate to form cellular bands to guide axon regrowth and release ECM molecules to support the growth potential of injured neurons.[35,47,48] Unlike inhibitory scarring in the CNS, Schwann cells and perineurial mesothelial cells produce abundant basal lamina to form regeneration-promoting channels that guide peripheral axon growth. Fibroblasts in the PNS secrete interstitial collagen surrounding the damaged nerves, which provides mechanical support to the injured axons and enables axon ensheathment.[35,49] Once Schwann cells reach the regenerating axons, they begin to remyelinate the newly formed axons.[48] Although inflammation promotes nerve regeneration after PNS trauma, failure to control or terminate the array of inflammatory reactions can lead to adverse outcomes that underlie neuropathology, such as pain and other syndromes that result after peripheral injury.

NEURAL REGULATION IN TRAUMA-INDUCED PAIN

Mechanical stimuli to the PNS are sensed and translated into electrical and biochemical signals by mechanosensitive afferents. Nociceptors, including medium-diameter myelinated Aδ fibers and small-diameter unmyelinated C fibers, encode painful information and can be activated by mechanical and/or chemical loading that exceeds the threshold for pain sensation, despite whether the force and deformation are applied directed at the nerves themselves or to the surrounding innervated tissue.[27,50−53] Those pain fibers have peripheral terminals that are embedded in tissues, such as joint capsules and the skin.[52,54] Their cell bodies are housed in the dorsal root ganglion (DRG) (Figure 2). Axons extending from the DRG to the spinal cord form the dorsal nerve root and synapse with neurons in the spinal dorsal horn (Figure 2).[27,50] Activation of nociceptors at the peripheral terminal triggers a complex cascade of electrophysiological events that convey the nociceptive messages from the periphery to higher order pathways in the spinal cord and the brain (Figure 2).[27,55,56] Transient noxious stimuli may lead to altered activity in the afferent neurons that is associated with pain, including lowered firing thresholds, increased firing rates, and persistent activation.[8,51,53] Excess mechanical loading can also induce the release of neurotransmitters, including the nociceptive neuropeptides substance P and calcitonin gene-related peptide (CGRP) produced by peptidergic C fibers.[27,57] In addition, neurons regulate their expression of inflammatory mediators and receptors for cytokines, neurotrophins, prostaglandins, and bradykinin, which are all released by glia and immune cells that reside or infiltrate the injured tissue.[27,58−60] All of these changes in sensory nerve fibers commonly result in decreased thresholds to thermal and mechanical stimuli in the local injury region, increase the excitability of output spinal neurons, and facilitate the transmission of pain information to the brain.[51,57,61]

Pain generation normally serves as a protective mechanism that signals the brain of existing or potential tissue injury.[55,62] Nociceptive signaling under physiologic conditions provides feedback to promote tissue adaptation and/or repair.[55,62] However, potentiation of nociceptive processing can develop into a chronic neurological disorder, deviating from the protective purpose of pain.[63,64] Aberrant nociception can cause pain sensitization that involves pathologic neuronal responses in the CNS and leads to spontaneous and evoked

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

behavioral hypersensitivity.[4,54,61,65,66] Mechanisms of neuronal regulation in pain are reviewed in greater detail here using two examples, namely, radicular neuropathic pain from a direct mechanical insult to neural tissue and joint pain from mechanical loading to the innervated spinal facet capsular ligament. These two examples, which involve both primary mechanical insults to tissues in the PNS and secondary complications across scales, demonstrate well the complex nature of neural injury and how multiscale techniques can be utilized to reveal therapeutic targets for treating pain.

Neuropathy from Nerve Root Compression. Although several regions of the cervical spine are susceptible to neck trauma, the cervical dorsal nerve root is especially vulnerable to injury and can lead to several different pain states including cervical radiculopathy.[4,67−71] Radiculopathy, a type of neuropathic pain, is characterized by a lesion to the nerve root due to its direct compression or impingement[72,73] and often results in pain or numbness that radiates down the arm or leg.[69,74] Preclinical in vivo studies suggest that even a transient compression of the nerve root can produce sustained mechanical and thermal hypersensitivity[9,13,16,17,75−79] as well as induce axonal degeneration in the root itself and profound macrophage infiltration.[9,13,17,80]

The specific signature of neuropathic pain resulting from direct mechanical loading of the nerve root depends on the specific mechanics of injury, including the type of load, the loading rate, and the duration of the compression.[8,9,16,75,81−84] These parameters all contribute to the extent of pain that is sustained long-after the initial trauma has occurred.[81] Rodent models of mechanical root injuries, specifically compression, provide evidence that the development of chronic pain is determined by separate load and duration thresholds.[7,15] While a transient compression of the cervical nerve root using 26 mN produces sensitivity lasting only for 24 h, any compressive load of the same duration above 38 mN induces sensitivity that lasts for at least 7 days.[16] Similarly, the duration of compression differentially determines pain responses, with a 15 min compression at a supra-threshold load (98 mN) producing sustained sensitivity, while a 3 min compression does not.[78] It is hypothesized that these mechanical parameters may induce pain by altering the gross structure of the nerve root itself. A nerve root compressed at the same magnitude, for 30 s or 3 min, recovers 88 ± 5% of its structural width, while one exposed to a 15 min compression recovers only 72 ± 13% of its original width,[78] suggesting there is a temporal component to the tissue's ability to recover its functional shape and that such timing may be related to the production of pain. Indeed, since neural tissue is viscoelastic, its mechanical response depends on these factors; it is not surprising that the physiologic responses leading to pain similarly depend on such loading factors. That report also found that the loss of structural recovery of the root after a 15 min compression may be due to, or produce, axonal disruption[78] and that a decrease in (or absence of) axonal transport may be associated with pain.[16] Another study found that a 15 min nerve root compression at a load of 32 ± 9 mN was sufficient to induce neuronal degeneration in the nerve root 7 days after injury.[9] Together, these reports suggest that the mechanical parameters necessary to alter neuronal structure in the nerve root are associated with the maintenance of pain.

In addition to inducing axonal damage in the root,[9,17] a painful root compression also alters neuronal activity and signaling more remotely in the spinal cord, where the injured afferents from the nerve roots synapse. At the cellular level, expression of the neuropeptides substance P and CGRP are decreased in the spinal dorsal horn up to 1 week after a compression of the nerve root that also produces pain but not other types of compressions.[9,16] This reduced expression of neuropeptides in the spinal cord may result from decreased synthesis in the DRG or disruption of the anterograde axonal transport of the afferents in the compressed nerve root,[85,86] both of which likely occur during mechanical loading and result in neuronal dysfunction and disrupted signaling.[87] Direct mechanical root injury also reduces peripherally evoked neuronal firing in the spinal cord, which also appears to depend on the specifics of mechanical loading, with a compression duration of 6.6 ± 3.0 min or longer being sufficient to reduce neuronal firing in the rat.[8] In contrast, if the root is compressed for a period shorter than that, the evoked neuronal signaling returns to precompression activity within 10 min after the compressive insult is removed and is insufficient to produce axonal damage in the root after injury.[8] Collectively, these studies establish that within a very short time of mechanical compression (here 6.6 min) axonal firing can be immediately modulated in the spinal cord. These studies also highlight that even a transient 15 min mechanical insult to the root, sufficient to induce pain, can also produce sustained neuronal dysfunction.

Similar work in animal models of loading to the lumbar nerve roots have found altered physiological responses. For example, tensile loading to the lumbar roots alters neuronal electrophysiology, including decreases in neuronal conduction velocity.[88,89] These reductions are strain-dependent; strains applied to the root below 10% do not alter electrically evoked compound action potentials, while strains between 10 and 20% decrease evoked action potentials, and strains greater than 20% render the neurons completely unresponsive.[88] Conversely, 7 days after painful root compression neuronal hyperexcitabilty or overactivity, hallmarks of chronic pain development and axonal damage, are evident in spinal cord neurons.[17,90]

Given the prevalence of neuronal dysfunction following nerve root injury, therapies targeting aspects of neuromodulation in the spinal cord have been increasingly considered as an approach to reverse alterations in neuronal activity.[91] Indeed, spinal cord stimulation (SCS) has been used to effectively reduce neuropathic pain or pain resulting from direct injury to neural tissue in both animal and human studies (Figure 2).[92−94] Conventional SCS administers either tonic stimulation in which there is a continuous pulse of electrical stimulation[95−97] or burst stimulation which uses periodic bursts of electrical stimulation.[98−100] Treatment with either a tonic or burst SCS paradigm attenuates behavioral sensitivity (i.e., pain),[101,102] with tonic SCS abolishing the decrease of spinal GABA that is typically observed for up to 14 days after painful nerve root compression.[102] Since neuromodulation is hypothesized to induce analgesia by activating inhibitory GABAergic interneurons,[103] attenuating this injury-induced decrease in GABA may explain the analgesic effects of spinal cord stimulation.

Along with disrupted axonal transport, increased glutamate release by afferents is a hallmark of peripheral nerve injuries as well as a critical mediator for the development of chronic pain.[104,105] Therefore, promoting extracellular glutamate uptake in neuropathic neural injury may be an effective strategy to reduce pain. Interestingly, administration of the drug ceftriaxone, which is known to upregulate the expression of the spinal glutamate transporter glial glutamate transporter 1

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

(GLT-1),[106] early (1 day) after a painful nerve root compression attenuates behavioral sensitivity as well as reduces neuronal hyperexcitability in the spinal cord (Figure 2).[90] Additionally, treatment with the neuroprotective drug riluzole, which blocks presynaptic glutamate release,[107] at the same time reduces structural abnormality of axons in the compressed root and abolishes pain for up to 7 days after injury (Figure 2).[17] Riluzole also increases the expression of spinal CGRP at day 7, suggesting that it may mitigate the axonal damage that occurs after a compression injury.[17] Taken together, these studies suggest that treatments that aim to reduce neuronal dysfunction and promote neuronal viability after a painful mechanical injury may not only reduce the pathologies created by mechanical loading but also be effective in reducing the subsequent development of pain.

Joint Pain from Facet Distraction. The facet capsular ligaments that enclose the bilateral spinal facet joints between adjacent vertebrae have been increasingly recognized as pain sensors due to their nociceptor innervation.[108−111] Supraphysiologic loading to the facet capsular ligaments can induce nociceptor activation and local inflammation, leading to pain.[20,24,59] To understand the mechanisms by which mechanical insults to these joints are translated into nociceptive signaling and pain, we and others have developed and integrated in vivo and in vitro models to assess neuronal regulation at both the afferents' peripheral and central terminals in response to facet trauma.

Previous animal and biomechanical studies provide evidence for different mechanical tolerances of the facet and its embedded afferents. In a caprine model, failure strains (72.9 ± 7.1%) of the facet capsule result in morphological abnormalities that are evident in its innervating axons, but subfailure strains between 10% and 50% are sufficient to activate mechanoreceptors in the facet capsule and induce prolonged afterdischarges.[20,24,52] Visible rupture in isolated cervical facet capsules is produced during tensile loading at strains which are greater than those that produce sustained pain in vivo.[12] Although subfailure tensile loading of the cervical facet joints in the rat induce persistent pain,[112−114] distracting the joint's capsule to failure produces only transient sensitivity, likely due to interrupted afferent signaling.[112] These findings not only highlight a need to more fully understand the complicated subfailure loading regime but also suggest the importance of joint afferents in regulating the production of pain.

Measuring the cellular and molecular changes in the rat DRG using biochemical assays reveals a host of possible afferent responses that contribute to the development and maintenance of nociception following facet trauma. For example, increased expression of substance P and modifications in glutamatergic transmission, such as upregulation of metabotropic glutamate receptor-5 (mGluR5) in nociceptive DRG neurons, parallel sustained pain after subfailure facet joint distraction.[113,114] Painful capsular stretch also stimulates the integrated stress response in the DRG as demonstrated by increased expression of a marker of endoplasmic reticulum stress response and upregulation of activating transcription factor-4 for long-term synaptic plasticity.[115,116] Robust nociceptive responses observed in undisrupted DRG neurons suggest that discontinuing peripheral signaling may be a potential therapeutic approach for treating persistent facet pain. This notion is further supported by the selective ablation of peptidergic neurons in the facet capsule using the neurotoxin saporin to ablate afferent in the joint before injury and the immediate blocking of those afferents using bupivacaine after capsule stretch, both of which prevent pain after a joint distraction that otherwise induces pain.[117,118]

Similar to neuronal responses after nerve root injury, nociceptive signals from afferents modulate neuronal excitability and dysregulate neuromodulator production in the spinal dorsal horn with traumatic joint pain. Hyperexcitability of dorsal horn neurons develops between 6 h and 1 day after joint injury and is still evident on day 7, paralleling the presence of sustained behavioral sensitivity.[119−121] These changes are accompanied by early upregulation of spinal substance P, modifications in the glutamatergic system including increased mGluR5 and altered expression of the glutamate transporter, and activation of other signaling molecules associated with neuroplasticity.[113,114] Antihyperalgesia drugs that attenuate neuronal excitability and signal transmission in the spinal cord have been shown to effectively reduce facet-mediated pain. For example, gabapentin, which is primarily used to treat seizures and neuropathic pain, significantly decreases the frequency of evoked firing of spinal neurons and attenuated mechanical hyperalgesia when injected via lumbar puncture prior to and 1 day after painful facet joint distraction in the rat (Figure 2).[121]

Painful facet joint injury, like neuropathy, initiates both local and widespread inflammation.[59,60,122,123] Recruitment and activation of glial cells as part of the immune response that may act as a protective mechanism for tissue and nerve recovery can also sensitize nociceptors via release of inflammatory mediators,[124] leading to aberrant neuronal activity and pain. The neurotrophic factor NGF increases in inflamed tissues to help nerve regeneration, but it is also a well-known neuronal sensitizer and contributes to osteoarthritic joint pain.[45,125,126] Its expression in the facet joint following painful tensile loading is increased as early as day 1 when pain has already been established.[59] Localized anti-NGF treatment using blocking antibodies immediately after facet joint injury prevents the development of neuronal hyperexcitability in the spinal cord as well as the onset of pain (Figure 2).[59] In addition to NGF inhibition, neuroprotective therapies may also be developed by targeting another neurotrophin, brain-derived neurotrophic factor (BDNF). The production of BDNF is upregulated in the DRG and the spinal cord after painful facet joint distraction.[60] Intrathecal administration of the BDNF-sequestering molecule trkB-Fc after pain is established from the fact that facet injury partially attenuates it.[60] Painful facet distraction also induces rapid upregulation of pro-inflammatory cytokines and prostaglandin E_2 (PGE_2) expression in the spinal cord and an increase in the production of PGE_2 receptor in the DRG early after injury.[58,122] Activation of astrocytes in the spinal dorsal horn is delayed and does not occur until a later time (day 7).[123] Suppressing such broad inflammation in the joint by intra-articular administration of a general nonsteroidal anti-inflammatory drug (NSAID), such as ketorolac, effectively alleviates facet-mediated pain in an animal model (Figure 2).[116,123] These findings, taken together, point to the importance of controlling post-trauma inflammation at the injury site to promote neural repair while mitigating pain.

MULTISCALE TREATMENT STRATEGIES FOR NEURAL INJURY

Defining the regulatory cascades of neural injury and understanding the mechanisms of axonal growth during

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

embryonic development inspire and guide treatment strategies for neural regeneration. However, since axon regeneration after injury is limited in the mature mammalian nervous system, especially in the CNS, the effects of neuronal injury from neurotrauma are exacerbated.[29,36,127] Regeneration is affected by both the intrinsic growth capacity and the extracellular environment of the neurons.[36,127] Current regenerative approaches utilize biomaterials and cell-based strategies or intervening in molecular pathways to either directly replace the injured neurons or modifying the microenvironment from one that inhibits growth to one that permits and even enhances it.[36,127] Neural tissue engineering approaches and pharmaceutical therapies have shown promise in enhancing regeneration and modulating inflammation in both the CNS and the PNS.[36,128] Specifically, scaffold materials containing live cells have been used to replace damaged tissue and cells (Figure 1).[129,130] Blocking growth-inhibitory pathways using pharmaceutical inhibitors suppress neurodegeneration at the molecular level (Figure 1).[131] Novel delivery routes involving on-target delivery of therapeutic compounds may be used to treat only the injured tissue (Figure 1).[132] Several treatment options, including bioactive scaffold and molecular inhibitors along with delivery methods, will be briefly discussed to demonstrate how multiscale therapeutic materials regulate the repair process after neurotrauma.

A particular promising tissue engineering approach involves the development of bioactive scaffolds, which use biomaterials in conjunction with various cell sources to rescue the damaged tissues and cells.[133,134] The specific properties of the biomaterials that are used in the scaffold not only determine the mechanical properties of the engineered constructs and optimize their use for appropriate integration with the tissue receiving the scaffold but also govern the microenvironment that regulates cell proliferation, differentiation, migration, and communication.[128,129,133] One challenge with neural regeneration is restoring connections to the desired targets over long distances.[135] The lack of robust and directed repair can limit the degree of functional recovery. In order to tackle this problem, bioactive scaffolds have been used to recapitulate neural cell migration and axon guidance.[128,133] By introducing the desired haptotactic, chemotactic, and mechanical cues, such as contact and soluble factor-dependent signaling and appropriate matrix stiffness, bioactive scaffolds have successfully promoted targeted axonal regrowth and integration with the host tissue.[128,129,136] One prototypical bioactive scaffold for guided nervous tissue reconstruction is microtissue engineered constructs with living embryonic neurons and glia.[128,134] In that construct, cells are embedded in miniature conduits, comprising an inner ECM core with bioactive ligands and a stiff hydrogel shell that provides structural support during transplantation.[128,134] Elongated and aligned axonal tracts and glial cells can simultaneously replace the cells that have been lost due to neurotrauma or neurodegenerative disorders and facilitate long-distance axon growth and pathfinding.[128,129,137] Such microscale tissue scaffolds display vigorous neuronal survival and axon extension that have been shown to mimic the neuroanatomy of brain tissue in vitro and may be used to restore neural circuitry in the CNS after injury with minimally invasive implantation.[128,129,134,136] Further, mechanical stimulation, like axon stretch, which simulates tension exerted by organism growth during development, can induce axon extension as great as 10 cm in 2−3 weeks in vitro.[138,139] Scaling up the microtissue constructs by embedding elongated axonal tracts in collagen matrices can be used to facilitate PNS regeneration.

Stem cells and progenitor cells are also promising cell sources used in bioactive scaffolds to introduce neurons and glial cells to the injured nervous tissue. Neural stem and progenitor cells are beneficial because they are multipotent, can produce sufficiently large number of cells, and may be genetically manipulated for therapeutic purposes.[140−143] Once transplanted at the injury site, neural stem cells and progenitor cells can respond to signals present in the damaged tissue and provide the cell types needed for repair and regeneration by differentiating into neurons and glial cells.[144,145] Unlike primary cells whose number is limited, stem and progenitor cells may generate large quantities of cells for therapy but do not present risks for tumorigenesis or robust immune reaction.[146−148] Neural stem-cell grafts and progenitor-cell transplants have been shown to promote neurogenesis in the brain and to facilitate functional repair.[144,149] For instance, multipotent neural precursors transplanted into the neocortex undergoing neuronal degeneration differentiate into neurons that morphologically resemble and replace pyramidal cells.[149] Following traumatic brain injury, injected embryonic neural progenitor cells can migrate to the damage location, provide trophic support and promote long-term motor and cognitive recovery.[144]

Although cell transplantation with bioactive scaffolds can facilitate neural regeneration and repair, this tissue engineering approach also has several drawbacks. Living cells, especially glia, can elicit robust immune responses.[150] Instead of using nonspecific immunosuppression, personalized grafts that are fabricated using host cells may attenuate such deleterious immune responses.[143,151] However, in some cases, generalized scaffolds are preferred due to issues such as cell source constraints, quality control, and commercialization considerations.[128,152] Certain stem cells are also immune privileged,[143,148] but aberrant regeneration due to increased regenerative capacity of the cellular environment and issues associated with graft delivery and degradation may not be avoided.[128,153] An alternative to transplantation is endogenous replacement by regulating the number and fate of stem cells in the CNS.[127,154,155] Despite whether bioactive scaffolds or self-repair strategies are used to replace cells, newly generated axons need to integrate fully and functionally with the remaining neural network in order to recover lost functions.

Although exacerbated inflammation raises questions about the safety of bioactive scaffolds, immune responses are crucial to neural regeneration following PNS trauma and must be carefully regulated in biomaterials-based therapies. The use of mesenchymal stromal cells (MSCs) in immunomodulatory and regenerative applications is rapidly increasing, not only because of its ability to differentiate into different cell types but also because of its paracrine effects.[156−158] MSCs can act as dynamic inflammatory modulators by releasing a host of biochemicals, such as neurotrophic factors and cytokines, in response to the local microenvironment, which may improve cell survival, recruit neighboring cells, and enhance cell-to-cell contact.[156−158] Allografts seeded with undifferentiated MSCs promote structural and functional restoration of injured ulnar nerves in rhesus monkeys.[159] MSC-derived Schwann cells have also been used as cell sources for transplantation therapy in median nerve injury to successfully achieve nerve restoration in monkeys, as demonstrated by behavioral, electrophysiological, and histological improvement.[160] The integrated use of MSCs

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

and carrier materials with suitable biochemical and mechanical properties is an attractive treatment strategy that may reduce the use of immunosuppressive drugs and achieve optimal recovery outcomes following neurotrauma.

Successfully restoring function after CNS injury not only depends on physical reconstruction by neural tissue transplants but also involves removal of growth inhibitors, delivery of neurotrophic factors, and manipulation of intracellular signaling.[127,161] Since the PNS has a much higher regenerative capacity compared to the CNS, peripheral nerve grafts have been used for neural tissue repairs after CNS injury.[161] Although peripheral nerve grafts provide an environment permissive for growth consisting of a supporting substratum and neurotrophic factors, axon extension may be limited by growth-inhibitory molecules produced by non-neuronal cells at the injury site and those present in the neurons themselves.[36,161−163] Inactivation or removal of growth inhibitors can facilitate the regenerating axons to grow out of the permissive substrate and migrate into the injured CNS.[36,161,164] For instance, Nogo-A is a myelin-associated oligodendrocyte-derived inhibitor for neurite outgrowth.[29] It can be blocked by the antibody IN-1 to enhance CNS plasticity and regeneration.[164]

The Rho-associated protein kinase (ROCK) is the major downstream effector of the signaling G-protein, RhoA, which can be activated by both mechanical and inflammatory cues.[165,166] Blocking ROCK in neurons via small molecule inhibitors has been shown to promote cell survival and axonal regeneration.[167−169] It has been identified as a potential therapeutic target for treating neurological disorders because its inhibition attenuates morphological abnormalities of neurons, reduces synaptic strength in vitro, promotes CNS repair and locomotor recovery following spinal injury, and alleviates pain after traumatic spinal nerve injury in vivo.[165,166,169−173] An alternative to eliminating growth inhibition by increasing the level of neurotrophins may be sufficient to drive the outgrowth of axons beyond the graft−host interface and to enable long-distance axon regeneration.[127,161] For instance, using transplanted fibroblasts that are genetically modified to produce BDNF facilitated regrowth of disrupted rubrospinal axons at the injury site through and around a hemisected spinal cord.[174] However, as noted above, excessive expression of inflammatory factors, such as BDNF, may exacerbate neuronal injury and further contribute to pathological conditions, especially pain.[60,175] Since neurotrophic factors contribute to both inflammation and neuroplasticity,[45,125,176] their production must be tightly regulated in order to balance their beneficial and adverse effects.

Although many possible drug treatments with specific cell-based mechanisms of actions have been identified to promote neural repair and alleviate chronic pain, often only a small amount of drug is actually delivered to the disease site.[177] The effectiveness of such treatments is compromised due to poor pharmacokinetic profiles, substantial off-target toxicity, and limitations with the administration route.[177−179] Using chronic pain as an example, current therapeutics often rely on passive targeting, in which enhanced permeability at the injury site and spinal cord allow for extravasation of drugs into the CNS.[180,181] Yet, those agents are rapidly cleared from the blood[182] and are not sufficiently delivered to the spinal cord where nociceptive processing occurs. Many chronic pain treatments in both human and animal studies have sought to improve efficacy by delivering therapies using intrathecal drug delivery systems.[90,121,183−188] Intrathecal drug delivery systems administer drugs directly into the spinal canal allowing direct access to cerebrospinal fluid, which improves on-target delivery as well as lowers the effective dose of drug needed.[183,185] However, such delivery systems, including intrathecal injections, are highly invasive and can result in complications like bleeding and infection.[189] Therefore, therapeutics are now incorporating active targeting in their formulations to facilitate localized delivery through minimally invasive administration routes.[132] Active targeting, also known as ligand-mediated targeting, utilizes antibodies or peptides to enable site-specific delivery and retention of drugs to areas that express the targeted ligand.[177,182] Target specificity is achieved by selecting molecular targets that are expressed *only* in pathological states and/or in injured tissue, thereby reducing the off-target effects.[190] Given that neuronal dysfunction often occurs not only in a peripheral injury site[9,13,16,17] but also in an area remote to that injury site in the spinal cord,[11,13,21] incorporating targeting ligands that are present in both of these regions may improve the efficacy of neuroprotective treatments.

THERAPEUTIC TARGETS AND MATERIALS FOR PAIN

Biomaterials-based strategies are under active research for treating chronic pain. Biocompatible hydrogels and therapeutic compounds are used to modulate the biomechanical and/or biochemical environment in an injured tissue to regulate inflammatory cell infiltration, glial activation, and morphological and functional changes in neurons, all of which are associated with pain.[17,80,165,191−194] The use of natural and synthetic substrates and compounds to target tissues, cells, signaling molecules, and genes in the nervous system is a powerful approach not only to elucidate the mechanisms involved in pain but also to help identify potential drug targets across different scales. Understanding the injury principals and identifying pain modulators also can serve to motivate the design of novel therapeutic materials and drugs. We specifically review how scaffold materials, inhibitory compounds, and active targeting may be used to regulate neuronal responses and to attenuate pain in animal models via three specific examples: (1) salmon-derived fibrin and thrombin to attenuate radicular pain, (2) integrin-targeted regulation of inflammation and mechanotransduction to mitigate innervated ligament pain, and (3) phospholipase A2 as nociceptive-specific targeting ligands for neuroinflammation. These applications were selected because they not only highlight how diverse, multiscale treatment strategies for neural injury can be utilized to alleviate pain but also emphasize different cellular and molecular mechanisms underlying mechanically induced pain.

Pain Mitigation by Salmon-Derived Coagulation Factors. Fibrinogen and thrombin are the two key proteins involved in the blood coagulation cascade. A biocompatible fibrin gel prepared from them can effectively prevent bleeding and support wound healing,[195] and can be used as a soft substrate to support neuron migration and regeneration.[151,191,193] The unique nontoxic polymerization mechanism allows cells to be encapsulated in the fibrin gels, making fibrin a suitable material for bioactive scaffolds.[191] Fibrin gels derived from fish products show great promise in promoting neuronal survival and regrowth and limiting glial activation because of the fact that teleost fish have naturally evolved enzymes that initiate distinct inflammatory responses from mammalian

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

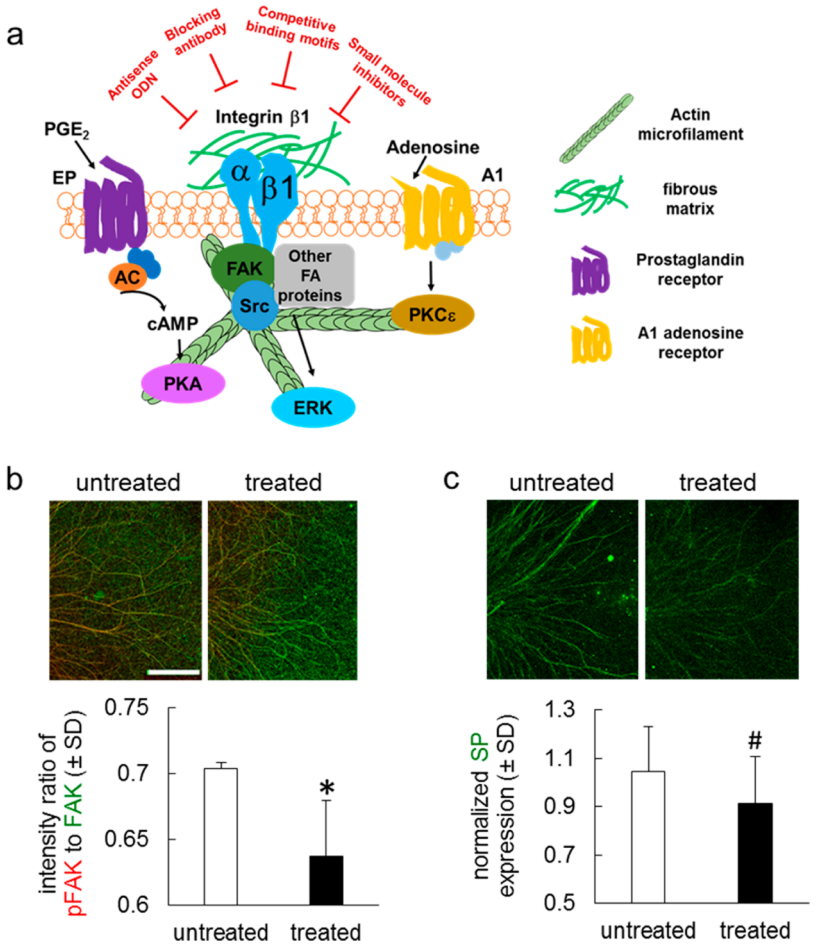


Figure 3. Involvement of β1 integrins in nociceptive regulation in neurons after painful loading. (a) Schematic showing potential β1 integrin-dependent cascades for the development of PGE_2-induced pain. The β1 integrin may contribute to inflammatory hyperalgesia via interactions with cell membrane receptors, the cytoskeleton, and a host of second messengers. Intervening in β1 integrin-dependent pathways with a variety of therapeutic compounds can effectively block PGE_2 hyperalgesia. (b) Representative images and quantification of the ratio of phosphorylated FAK (pFAK; red) to total FAK (green) after gel stretch simulating painful strains show FAK phosphorylation decreases (*p = 0.03) with integrin inhibition. (c) Expression of the nociceptive neuropeptide substance P (SP) exhibits a similar difference decreased expression in axons after stretch (#p = 0.04) with integrin inhibition. The scale bar in b represents 500 μm and applies to all images in panels b and c.

species.[191,192,196] Without additional growth factors, fibrin alone has been used to facilitate neural repair, partially due to its mechanical properties which support neurite extension without eliciting robust glial responses.[191−193] Moreover, the structure and stiffness of the fibrin gel can be modulated by varying the reaction conditions during polymerization, leading to the formation of a variety of soft hydrogels that are suitable for physiological applications.[195] Fibrin gels derived from mammalian sources degrade rapidly and present the risk of infection of blood-borne pathogens and clotting disorders.[195,197,198] The limitations of mammalian fibrin have led to the preparation of fibrin from other species, such as salmon. Because of the evolutionary differences, fish and mammals have different components that are involved in blood clotting and associated with inflammatory responses.[196] As such, salmon-derived thrombin, an enzyme that converts fibrinogen to fibrin, activates different cell signaling cascades and reduces glial transcription of pro-inflammatory cytokines as compared to its human counterparts.[80,194,196] Further, in comparison to mammalian fibrin, salmon fibrin displays a slower degradation profile, starts clotting at a lower temperature, and results in greater neurite extension in vitro.[193,199,200] When injected into the lesion site following SCI in the rat, salmon fibrin provides improved functional recovery as compared to human fibrin,[201] which suggests that it may be an effective therapeutic biomaterial for treating neural injury.

Given the neuroprotective effects of fibrin, we investigated if the unique anti-inflammatory clotting factors in salmon fibrin can reduce the pain that is induced by mechanical trauma after nerve root compression in a rat model. Salmon fibrin, which was administered at the nerve root immediately after compression that normally produces pain, both mitigated pain for at least 7 days and reduced the associated inflammatory responses that the injury produces at that time.[194] In particular, salmon fibrin treatment mildly decreased the extent of macrophage infiltration that is typically evident at the injured nerve root and decreased the activation of spinal astrocytes that also develops after injury.[194] These findings suggest that direct application of salmon fibrin onto the injured nervous tissue in a radiculopathy model has analgesic effects, likely via altering (or preventing) the typical inflammatory response that accompanies this tissue injury.

Previous studies demonstrate a role of thrombin, a key component of fibrin, in mediating various cellular cascades

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

responsible for neuronal health and pain.[202,203] Like salmon fibrin, salmon thrombin is neuroprotective, anti-inflammatory, and antinociceptive.[80,194,204] Treating a painful nerve root compression with salmon thrombin also reduces macrophage infiltration and further preserves myelination of neurons at the compressed root, as well as preventing the hyperexcitability of spinal neurons that is associated with pain and fully attenuating pain.[80,194] In contrast, treatment with *human* thrombin has no such beneficial effects.[42] Prior evidence suggests that whether mammalian thrombin intensifies or transiently reduces pain likely depends on the activation rate of the protease-activated receptor-1 (PAR1). PAR1 is expressed on neurons and astrocytes and can be activated by thrombin,[205−208] leading to increased release of nociceptive neuropeptide and inflammatory cytokines.[205,207−209] Using kinetic analysis and clotting tests, salmon thrombin activates a PAR1-derived peptide more weakly and retains higher enzymatic activity for clot formation with fibrinogen compared to human thrombin.[80] Salmon thrombin also has been shown to regulate the nociceptive responses via protein C-mediated pathways.[204] It protects vascular integrity by turning endothelial-bound protein C into activated protein C when bound to endothelial thrombomodulin.[210−212] By stabilizing vascular integrity, salmon thrombin also prevents the disruption of the blood−spinal cord barrier, thereby reducing inflammation after painful nerve root compression.[204] In contrast, mammalian thrombin directly activates PAR1 on endothelial cells and increases the vascular permeability.[213,214] Protein modeling uncovered a highly divergent sequence between human and fish thrombin, the deletion of which enhances the interaction of thrombin and protein C.[204] Taken together, findings obtained using techniques across scales from whole animal experiments to protein analysis highlight the material advantages, including anti-inflammatory and analgesic capabilities, of salmon-derived biologic agents. The efficacy of direct administration of fibrin and thrombin to the injury site points to a potential role of salmon-derived coagulation factors in treating neuropathic pain by regulating the neuronal health in association with reduced local inflammation (Figure 2).

Nociceptive Role of β1 Integrins. Integrins are transmembrane receptors that are expressed on many types of cells, including primary afferent neurons.[215,216] Integrins, particularly the β1 integrin, have been shown to play a role in sensitization of nociceptors and can initiate hyperalgesia (i.e., increased behavioral sensitivity from stimuli that usually induce pain) in rodent models of inflammatory and neuropathic pain.[217,218] The α and β integrin subunits can interact with both the ECM and the neuronal cytoskeleton, leading to bidirectional signaling across the cell membrane.[216] Binding to ECM ligands can activate integrins and trigger cascades of intracellular events.[216] On the other hand, integrins also can be activated or primed by intracellular signals from activation of other cell membrane receptors.[217,218]

Interfering with integrin signaling in the rat prevents the development of pain that is induced by intradermal injection of the inflammatory mediator PGE_2.[217−219] The β1 integrin-dependent signaling can be reduced or inhibited by intradermal injection of laminin fragments or functional-blocking antibodies and intrathecal injection of antisense oligodeoxynucleotides (ODNs) (Figure 3a).[217−219] Blocking or knocking down β1 integrins prior to PGE_2 injection via any of those methods has been shown to attenuate PGE_2-induced pain.[217−219] Further investigation into the interactions between second messenger cascades and integrin subunits in primary afferents suggests that PGE_2 leads to short-lasting mechanical hyperalgesia (i.e., pain) through β1 integrin-dependent AC/cAMP/PKA pathways (Figure 3a).[218] In rats primed by chemical carrageenan or a PKC_ε activator, PGE_2 injection leads to prolonged behavioral hypersensitivity.[219−221] Different from short-lasting pain induced by PGE_2 alone, sustained hyperalgesia after PGE_2 injection in primed rats depends on adenosine-mediated PKC_ε pathways and intact cytoskeleton (Figure 3a).[219−221] Using integrin β1 antisense ODNs, the prolongation of sensitivity induced by PGE_2, via the activation of A1 adenosine receptors, can be eliminated in rats that are previously treated with a selective PKC_ε activator.[219] In addition to PGE_2, other inflammatory mediators, such as NGF, adrenaline, and epinephrine, can also induce pain but through different second messenger signaling pathways.[125,217,218] The β1 integrin is nonselectively involved in several pain-related signaling cascades, including the PKA, PKC_ε, and the MAPK/ERK pathways, and its inhibition can prevent pain induced by various inflammatory factors.[218,219] Antisense knockdown of the β1 integrins has also been shown to eliminate neuropathic pain induced by systemic administration of the cancer chemotherapy agent taxol.[217] These findings suggest that the β1 integrin plays an important role in many neuronal pathways that modulate pain initiation from chemical irritation.

Integrins may also mediate mechanically induced pain, due to their involvement in mechanotransduction. The peripheral afferent terminals that innervate peripheral tissues may interact with the ECM and neighboring cells and sense external tissue loading via integrin-mediated focal adhesion. Prior studies of the hairy skin of the rat revealed $\alpha2\beta1$ integrins present on afferents in the skin.[222] Further, ex vivo stretch of rat skin produced neural excitation, which was prevented by inhibiting $\alpha2\beta1$ integrins with function-blocking antibodies before loading.[223] Those findings suggest that integrins may trigger activation of sensory neurons during trauma and that the peripheral terminals of pain fibers are potential sites for mediating mechanical transduction. To test whether tissue loading initiates nociceptive signaling of embedded afferent neurons via integrin-mediated pathways, we developed an in vitro neuron-collagen gel construct system that mimics the innervation of peripheral tissues.[224] Preincubation of the constructs with RGD peptides, which are known to inhibit β1 integrin-dependent epinephrine hyperalgesia,[217] significantly reduces the phosphorylation of focal adhesion kinase (FAK) (Figure 3b). Altered FAK activation indicates changes in integrin signaling because phosphorylation of FAK is a key step along the signal transduction pathways triggered by integrins.[225] Further, for gels undergoing tensile loading to strains that are sufficient for afferent activation, axonal expression of substance P, which is increased in sensory neurons after painful loading of tissues they innervate,[113] is significantly lower after treatment with integrin inhibitors compared to that of untreated gels (Figure 3b). Our findings support the involvement of integrins at the cell−matrix interface in the sensitization of nociceptors from supraphysiologic tissue loading. These effects are likely due to mechanotransductive signaling, but additional studies are needed to further investigate if and how various integrin-dependent pathways interact with different nociceptive molecules and affect neuronal activity after tissue loading using cell signaling assays and electrophysiological recording. Nonetheless, this study points to the β1 integrin as a potential pain mediator in trauma.

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

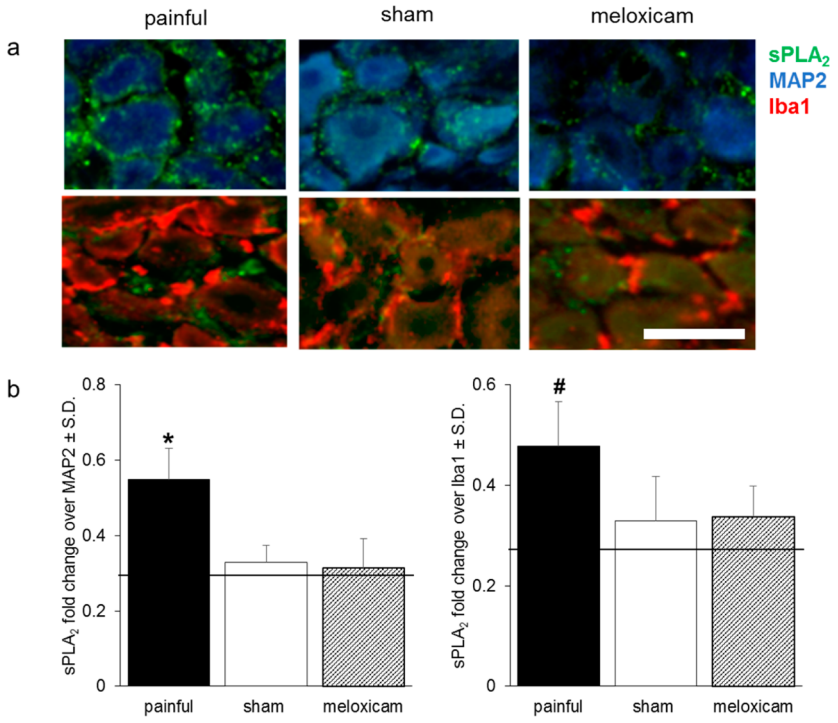


Figure 4. Expression of inflammatory $sPLA_2$ in the DRG is associated with the presence of pain. (a) Representative images of the DRG after a painful root compression, sham control surgery, or painful root compression with NSAID treatment by meloxicam. MAP2 labeling of neurons is consistent in all groups, while there is more Iba1 and $sPLA_2$ labeling after painful compression compared to that in the sham and meloxicam treatment. The scale bar is 50 μm and applies to all images. (b) Quantification of $sPLA_2$ expression normalized to MAP2 expression is significantly increased in the painful DRG over both sham (*p = 0.007) and meloxicam-treatment (*p = 0.003). Expression of $sPLA_2$ in microglia also significantly increases in the painful group over both the sham (#p = 0.006) and meloxicam treatment (#p = 0.003). Lines on both plots represent the relative $sPLA_2$ expression in normal naïve DRGs.

As mentioned above, painful stretch of the capsular ligament of the spinal facet joint is a complex mechanical injury involving activation of joint nociceptors by excessive tissue loading. That injury is confounded by inflammatory responses, including increased inflammatory mediators, such as NGF, in the facet joint and upregulation of the PGE_2 receptor in primary sensory neurons.[58,59] On the basis of the mechanical nature of loading during the facet injury and the fact that integrin inhibition attenuates NGF- and PGE_2-induced pain,[226] it can be hypothesized that integrins may serve as a therapeutic target for joint-mediated pain. Targeting integrin subunits on afferents at the facet joint by intra-articular delivery of monoclonal antibodies or small molecule inhibitors may attenuate pain by blocking both the mechanotransductive and inflammatory pathways that depend on integrin signaling. Yet, the expression of different integrin subunits in afferent terminals innervating the facet joint remain unknown, and whether and through which mechanisms integrin inhibition regulates facet-mediated pain require further investigation.

Phospholipase A2 as a Targeting Ligand for Neuroinflammation. Development of therapeutics for chronic pain remains difficult since regulation of nociceptive transmission often requires treatment at the site of injury and also in the DRG,[227,228] where peripheral pain processing is mediated. Although active targeting of therapeutics has been explored to facilitate their localized delivery for cancer treatment,[132,177,190] such targeting of pain therapeutics first requires identifying nociceptive-specific targeting ligands that are present. Given the contribution of neuroinflammation to the development and maintenance of chronic pain,[58,59,75,78,79,90,122] ligands specific to neuroinflammation or aspects of that cascade may facilitate localized delivery to the site of nociceptive regulation.

A subfamily of the phosopholipase-A2 enzyme, secretory phospholipase A2 ($sPLA_2$), can specifically recognize and hydrolyze the sn-2 bond of glycerophospholipids, releasing free fatty acids such as arachidonic acid (AA).[229] These hydrolysis products are well-known mediators of inflammation and tissue damage;[229,230] specifically, the hydrolyzed free fatty acids are used in the cyclooxygenase pathways to produce prostaglandins and other inflammatory molecules.[230] Additionally, increased $sPLA_2$ expression has been reported in many different persistent pain states that are characterized by inflammation, including intervertebral disc degeneration[231] and spinal cord injury.[232] After a peripheral neuropathic injury such as constriction of the sciatic nerve, $sPLA_2$ expression increases in both the DRG and the spinal cord.[233] Additionally, $sPLA_2$ is constitutively expressed in neurons and immune cells,[229,230] with increased expression induced upon stimulation with pro-inflammatory cytokines.[233,234] Taken together, these studies suggest that $sPLA_2$ may have an important role in pain processing. Accordingly, leveraging an elevation in $sPLA_2$ to be self-regulated targeting ligand for therapeutics may provide a potent treatment for pain.

In the same rat model of painful nerve root compression as referred to above about salmon-derived biomaterials, $sPLA_2$ expression increases in the DRG after painful compression (Figure 4). Compared to a sham surgical control, $sPLA_2$ normalized to the neuronal (microtubule-associated protein 2; MAP2) and microglial (ionized calcium binding adaptor molecule 1; Iba1) markers was significantly increased only after

DOI: 10.1021/acsbiomaterials.7b00012
ACS Biomater. Sci. Eng. 2017, 3, 2744−2760

painful compression. Interestingly, when pretreating the nerve root compression with the specific cyclooxygenase inhibitor meloxicam, pain was prevented[235] as well as the increase in $sPLA_2$ expression in the DRG, maintaining it at sham control levels (Figure 4). In this case, *systemic* administration of meloxicam prevented the upregulation of $sPLA_2$ in the DRG, suggesting that increases in $sPLA_2$ following nerve root compression not only occur in a pain-specific manner but also may contribute to the initiation of nociceptive cascades. Several in vivo studies have shown that administration of phospholipase A2 inhibitors attenuate pain[236,237] and that $sPLA_2$ inhibition in vitro prevents the release of the nociceptive neuropeptide substance P from DRG neurons stimulated with the inflammatory cytokine IL1β.[234] These studies provide evidence supporting $sPLA_2$ as a targeting ligand for pain. Additionally, given that $sPLA_2$ increases in the sites of nociceptive processing like the DRG[233,234] and spinal cord,[230,232] using it as a targeting ligand for pain therapeutics could provide improved localized delivery and facilitate greater effectiveness by reducing off-target toxicity and the overall dose of drug that may be needed.

SUMMARY

Mechanically induced pain from direct or indirect neural injury involves complex regulation of the nervous system at different length scales. Both direct and indirect insults to nervous tissue can disrupt tissue homeostasis, changing the mechanical and chemical environment of neurons. Depending on the injury severity and the cellular composition and pre-existing history of the injured tissue, an altered microenvironment can trigger a host of neuronal and inflammatory responses that have either protective effects or exacerbate the injury leading to pathological disorders like pain. Understanding the interplay between injury biomechanics and multiscale neural responses, ranging from tissue damage to altered gene expression, is essential to designing effective treatment strategies for pain.

We reviewed in detail how different biomaterials approaches may be used to modulate the cascades leading to pain. For example, salmon fibrin may be an ideal novel biomaterial for modulating neuroinflammation and attenuating radicular pain (Figure 2). Integrin-mediated neuronal regulation is also a potential therapeutic target for intervening in inflammation and mechanotransduction associated with traumatic joint pain (Figure 3). Lastly, $sPLA_2$ targeted ligand chemistries also show promise in mediating cellular responses via controlled release of drugs. Through those examples, we demonstrated how mechanistic discoveries can be used to guide therapeutic intervention and how various techniques may be utilized to target different pathways associated with pain. Bioactive scaffolds and therapeutic compounds delivered via novel routes are promising approaches to replacing the injured neurons, promoting recovery and intervening cell signaling cascades (Figure 1). Recent studies have identified several biomaterials and inhibitory compounds that have unique anti-inflammatory, neuroprotective, and analgesic effects (Figure 2), and are potential treatment strategies for traumatic pain. Although a more consistent cellular and molecular schema for neural injury is emerging and informing the design of therapeutic materials and drugs, much work is still needed to fully understand the neuronal regulations in pain from mechanical insults.

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S.Z., S.K., and B.A.W. designed the research and contributed to the writing and editing of the manuscript. S.Z., J.L., and S.K. performed the experiments shown in Figures 3 and 4.

Funding

Funding support was provided by the National Institutes of Health (U01EB016638), the Catherine Sharpe Foundation, the Cervical Spine Research Society, and an AMTI Force and Motion Scholarship, as well as an NSF training grant.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge Harrison Troché for assisting with analysis of some of the data presented in Figure 4.

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