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Spinal Interleukin-10 Therapy to Treat Peripheral Neuropathic Pain

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Abstract

Introduction—Current research indicates that chronic peripheral neuropathic pain includes a role for glia and the actions of proinflammatory factors. This review briefly discusses the glial and cytokine responses that occur following peripheral nerve damage in support of utilizing anti-inflammatory cytokine interleukin-10 therapy to suppress chronic peripheral neuropathic pain.

Spinal Non-viral Interleukin-10 Gene Therapy—IL-10 is one of the most powerful endogenous counter-regulators of pro-inflammatory cytokine function that acts in the nervous system. Subarachnoid (intrathecal) spinal injection of the gene encoding IL-10 delivered by non-viral vectors has several advantages over virally-mediated gene transfer methods and leads to profound pain relief in several animal models.

Non-viral gene delivery—Lastly, data are reviewed that non-viral DNA encapsulated by a biologically safe co-polymer, poly(lactic-co-glycolic) acid (PLGA), thought to protect DNA, leads to significantly improved therapeutic gene transfer in animal models, which additionally and significantly extends pain relief.

Conclusions—The impact of these early studies exploring anti-inflammatory genes emphasizes the exceptional therapeutic potential of new biocompatible intrathecal non-viral gene delivery approaches such as PLGA microparticles. Ultimately, ongoing expression of therapeutic genes are a viable option to treat chronic neuropathic pain in the clinic.

Keywords

Plasmid DNA; gene therapy; PLGA microparticles; allodynia; rat

Introduction

Chronic peripheral neuropathic pain is often associated with injury and inflammation of peripheral nerves [1], which may also involve several types of glial cells that critically contribute to signaling mechanisms leading to pathological pain states [2, 3]. A full discussion of neuronal mechanisms underlying nociceptive transduction that ultimately

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results to pain perception, as well as the factors that may mediate the transition from acute to pathological pain have been reviewed previously [4, 5]. In this mini-review, a brief and simplified description of neural and glial involvement in pain-related processing will provide a framework to support the rationale for applying the anti-inflammation cytokine gene, interleukin-10 (IL-10), as a clinically relevant gene therapy to alleviate chronic peripheral neuropathic pain.

In brief, abnormal neuronal activation following peripheral nerve injury can occur in any one or a number of pain-relevant neuroanatomical sites: 1) the peripheral or central nerve terminal, 2) the dorsal root ganglia (DRG) that house the sensory neurons of the body, 3) the trigeminal ganglia that contain most of the sensory neurons of the head (face, eyes, nasal and oral cavities, and other structures), 4) pain projection neurons in the dorsal horn of the spinal cord or within the trigeminal sensory nucleus of the brain stem, and 5) numerous brain areas [6-8]. When nociceptive signals are enhanced and become chronic (greater than 3-6 months) [9, 10], the protective mechanism that normal acute pain serves for recuperation and wound healing is no longer intact. The prevalence of neuropathic pain (cancer and non-cancer pain) in the general US adult population is estimated at 7-8% [11, 12], reflecting a need to explore novel approaches for the development of therapeutics to treat such pain. Research of the past two decades reflects a significant advancement in understanding the biochemical and cellular mechanisms underlying pathological pain. Indeed, modern views of pain processing are emerging which include a critical role of non-neuronal glial cells strongly implicated in the pathogenesis of neuropathic pain processing in the sensory ganglia and central nervous system [2, 3, 13].

While spinal glial cells are documented to play critical roles in the development of peripheral neuropathic pain [3, 14, 15], a number of more recent reports demonstrate that brainstem glia within the medulla are also critical in mediating orofacial pain conditions [16]. Additionally, satellite glial cells in the sensory ganglia at spinal and supraspinal levels are major contributors to pathological pain problems [3, 16]. For example, glia are capable of contributing to persistent neuronal excitatory signaling by responding to and releasing a variety of factors that include nucleotides (adenosine 5'-triphosphate; ATP) [17-21], proinflammatory cytokines like interleukin-1 β (IL-1 β or tumor necrosis factor- α (TNF- α) [15, 22], as well as chemokines (chemo-tactic signaling factors that induce peripheral immune cell trafficking) such as CCL2 (also known as monocyte chemoattractant protein 1; MCP1) [23-27]. It is notable that these glial products further stimulate the release of the classic pain-related neurotransmitters, substance P and glutamate from neurons [28-30]. Neurons, astrocytes and microglia express receptors for a variety of immune-related signaling molecules like IL-1 β , TNF- α and CCL2, enabling each factor to further stimulate glial IL-1 β and TNF- α release leading to a positive *feed-forward* excitation of both neurons and glia.

Interleukin-10

IL-10 is a pleiotropic cytokine. Within the central nervous system, a number of reports support that IL-10 is neuroprotective, as evidenced by enhanced *in vitro* cell culture survival of embryonic or immature cortical neurons, retinal ganglion cells, cerebellar granule cells, spinal cord neurons [31-34], or in hippocampal slice preparations from juvenile rats [35] following excitotoxicity. One method used to directly examine IL-10 effects on neuronal survival is by viral transfection of the exogenous IL-10 gene in cultured spinal cord embryonic neurons [36, 37]. In vivo local spinal release of IL-10 is achieved by similar methods of virally-mediated IL-10 gene transfer, which results in improved neuronal survival of the anterior quadrant of the spinal cord following spinal cord injury in rats [37]. In addition to neuroprotective roles of IL-10, anti-inflammatory intracellular actions also

result following IL-10 receptor binding. The IL-10-mediated activation of the Janus tyrosine kinases (e.g. JAK1 and Tyk2) results in recruitment of the Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) to the IL-10 receptor subunit, IL-10R1 (for review [38]). Nuclear translocation of STAT3 results in STAT3 binding to various promoter elements of genes including IL-10 itself, ultimately leading to anti-inflammatory activity. For example, IL-10 results in decreased nuclear factor- κ B (NF- κ B) activity that leads to decreased production of immune-response genes necessary for proinflammatory cytokine (e.g. IL-1 β and TNF- α) and chemokine (CCL2) production by macrophages, dendritic cells, natural killer cells, and Th-1 and Th-2 cells. Indeed, the most-widely characterized biological function of IL-10 is its ability to suppress immune responses.

IL-10 signaling occurs through its class II receptor that functions as a dimer. The IL-10 receptor (IL-10R) is composed of 4 chains, two IL-10R1 type chains and two IL-10R2 type chains [39]. The IL-10R1 unit critically mediates high affinity IL-10 binding and signal transduction, while IL-10R2 is required for signaling only [40]. Based on studies examining functional cellular responses to IL-10, the expression of the IL-10R (both IL-10R1 and 2) is most-widely characterized in cells of the hematopoietic lineage [40, 41], such as monocytes, macrophages, dendritic cells, B cells, T cells, neutrophils and natural killer cells. Interestingly, while a recent report characterized IL-10R1 in embryonic spinal cord neurons following transgene IL-10 stimulation in neuronal cell culture [34, 36], postnatal IL-10R1 is expressed in astrocytes, microglia/perivascular microglia, oligodendrocytes and endothelial cells, but not neurons, in the intact brain [42]. In the adult brain and spinal cord, the cellular pattern of IL-10R expression is similar to that observed in the immature postnatal central nervous system, with expression observed in astrocytes, microglia/macrophages and oligodendrocytes under pathological conditions [43-45].

The anatomical expression pattern of IL-10 protein and mRNA overlaps pain-relevant signaling regions, suggesting that an additional biological action of IL-10 may be to control glial and immune cell-mediated pathological actions on neurons. For example, IL-10 protein and mRNA is observed in peripheral nerve segments, DRG, spinal cord and the brain in a temporally-dependent manner following peripheral nerve damage that produces neuropathies in animal models. In peripheral nerve segments following chronic contraction injury (CCI) of the sciatic nerve, a widely used rodent model of peripheral neuropathy [46], or in a model of sciatic nerve transection, significant initial increases in nerve segment IL-10 protein and mRNA expression are observed by 24 hours that persist for 3 days following injury [47-50]. However, IL-10 mRNA and protein levels dramatically drop by 1 and 2 weeks after injury, and at other timepoints, IL-10 mRNA levels are significantly less than control levels [50]. Similarly, decreases in IL-10 protein are observed 2 weeks after unilateral injury from nerve segments [47]. An interesting aspect of IL-10 mRNA expression in contralateral nerve segments is the observed biphasic expression that occurs during a 42-day timecourse, with mRNA levels frequently measured well-below control levels [50]. Thus, these transient decreases in IL-10 expression may allow for opportunistic periods when proinflammatory pain-related signaling is established to drive pathological neuronal signaling.

As with peripheral nerve analysis, biphasic DRG IL-10 decreases in protein levels occur during a 2-week time course following CCI, with significant decreases that persist for 2 weeks compared to sham-operated controls values [51, 52]. In a separate report that also utilized CCI as a rodent model for mononeuropathy, quantification of immunohistochemical IL-10 detection in small diameter neurons in the DRG revealed that IL-10 immunoreactivity decreased by 50% compared to control-treated animals [53]. While compelling, these are the only data revealing immunohistochemical evidence of adult DRG neuronal IL-10 protein expression in vivo. More recently, IL-10 protein expression levels were increased in the

brain following pathological conditions, with expression observed in astrocytes, microglia and endothelial cells, but not neurons [42]. In a separate study, the spinal distribution of IL-10 was also reported in non-neuronal cells [45]. One possibility for these reported differences may be that DRG neurons are unique regarding IL-10 expression, supporting an ideal anatomical location relevant to satellite glial pain-related signaling.

As noted above, IL-10 suppresses proinflammatory cytokine production. However, IL-10 exerts additional control over IL-1 β and TNF- α actions by preventing activation of a number of intracellular signaling molecules such as p38 mitogen-activated protein kinase (p38-MAPK) important for glially-mediated pathological pain in animal models [54, 55]. In addition, IL-10 destabilizes IL-1 β and TNF- α mRNA, and it counter regulates the actions of IL-1 β and TNF- α by increasing IL-1 receptor antagonist & TNF decoy receptors. Thus, a comprehensive suppression of at least IL-1 β and TNF- α production and signaling can be achieved through the actions of IL-10 ([56], for review).

Based on the large body of evidence that IL-10 regulates neuroinflammatory processes in pain-relevant regions of the nervous system, it appears that IL-10 is a good candidate cytokine to control glial proinflammatory products that act to enhance pain transmission. In several animal models that lead to pathological pain-like behaviors, lumbosacral intrathecal delivery of recombinant IL-10 protein blocks the onset of light touch mechanical allodynia (pain associated with non-painful stimuli) induced by either dynorphin [57] or peri-sciatic phospholipase A2 [58]. Notably, allodynia in both of these models is mediated by spinal IL-1 β . Further, spinal cord excitotoxic injury associated with increases in spinal cord glial activation and proinflammatory cytokines, leads to pathological pain-like responses that are blocked by IL-10 [59-63]. In an animal model of chronic orofacial inflammatory pain, hyperalgesia is significantly attenuated by local intra-medullary application of IL-10 [64]. Prior characterization of this inflammatory model revealed concurrent increased astrocyte and IL-1 β reactivity in the medullary dorsal horn transition zone [22]. Together, these studies support a therapeutic role for IL-10 in suppressing glial-cytokine-mediated pain.

While short-term disruption of proinflammatory cytokine action and pathological pain states by IL-10 is sufficient to identify a potential anti-inflammatory therapeutic, prolonged disruption is critical for clinical chronic pain control. In chronic pain patients, several studies indicate suppressed IL-10 functions. In serum from patients suffering from chronic widespread pain (e.g. fibromyalgia) compared to those without chronic pain, significant increases in proinflammatory cytokines levels with corresponding decreases in anti-inflammatory (IL-10 and IL-4) cytokines were identified [65]. People with complex regional pain syndrome also revealed increased IL-1 β and IL-6 (often characterized as a proinflammatory cytokine) in CSF [66]. Thus, ongoing negative feedback suppression of proinflammatory cytokine activity with anti-inflammatory cytokines is a desirable clinical approach.

Intrathecal non-viral pDNA Gene Delivery

Given sustained suppression of proinflammatory cytokine action could be beneficial in people with chronic neuropathic pain conditions, exploring long-duration expression of anti-inflammatory cytokines like IL-10 via transgene delivery may be a promising and novel approach. Several gene therapy vector approaches for neuropathic pain control are being pursued for directed delivery to the DRG and spinal cord dorsal horn regions [67-70]. Gene transfer using non-viral naked plasmid DNA (pDNA) is the least effective method to transform host cells with therapeutic genes of interest [71]. The inefficiency is believed to be due to the multiple barriers that free pDNA must overcome for gene activation within the nucleus to occur. The cell wall membrane, intracellular and lysosomal degradative enzymes,

and the nuclear envelope all provide significant obstacles. Despite these hurdles, pDNA transgene delivery has significant advantages because it avoids the potential dangers that can occur with viral vectors, as viral vectors can be immunogenic and may result in undesirable transgene insertion, (e.g. spontaneous mutagenesis). Further, non-viral pDNA is relatively inexpensive to manufacture. In animal studies, lumbosacral intrathecal delivery of non-viral pDNA encoding the IL-10 gene leads to enduring transgene expression and long-duration suppression of allodynia from peripheral neuropathy [72] that significantly outlasts behavioral therapeutic efficacy following intrathecal viral-vector IL-10 gene transfer [73, 74].

The intrathecal compartment for targeted gene transfer offers an anatomical advantage for localized subarachnoid transgene delivery for several reasons. First, low numbers of surveillance phagocytes such as macrophages, dendritic cells, fibroblasts, and some microglia are present in the meningeal membranes that surround the intrathecal region [75-77]. Glia and other phagocytes in the meninges are capable of responding to intrathecally delivered material like pDNA by increasing phagocytic activity. Second, pain relevant sensory neuron terminals are located in close proximity to the intrathecal meninges. Thus, despite the typically low levels of transgene expression produced by non-viral gene transfer, non-viral pDNA targeted to the spinal cord has resulted in successful long-duration transgene expression [78] thought to be mediated by responsive intrathecal phagocytes.

The Sensitization Period

In studies to date, intrathecal delivery of naked pDNA encoding the IL-10 gene (pDNA-IL-10) produces prolonged reversal of neuropathic pain [72, 78, 79]. While intrathecal gene therapy to treat neuropathic pain is not unique [80-86], non-viral pDNA-IL-10 gene delivery is a more recent and novel approach. In studies to characterize non-viral pDNA-IL-10 therapeutic efficacy, a widely-used rat model of ongoing peripheral mononeuropathy was utilized (sciatic nerve chronic constriction injury [46]) because it produces prolonged, stable mechanical allodynia that allows for repeated assessment of sensory thresholds for 3 months and is mediated by spinal proinflammatory cytokines [72, 87].

A period of subarachnoid sensitization is a critical component of optimal pDNA-IL-10 gene transfer, which was discovered while exploring optimal pDNA-IL-10 doses for enduring pain reversal [72, 87]. The sensitization period requires two, sequential subarachnoid injections of non-viral pDNA to produce enduring suppression of allodynia from chronic constriction injury. The initial subarachnoid injection of pDNA is thought to sensitize the subarachnoid region for enhanced transgene uptake upon the second injection of pDNA encoding IL-10. Intriguingly, the sensitization period is discrete because the 2nd subarachnoid pDNA injection does not lead to long-duration suppression of allodynia when injected less than 5 hrs, or longer than 72 hrs, after the first injection [72, 87]. Thus, the optimal inter-injection interval is ~ 5 -72 hrs [87]. Mechanical allodynia [72, 78, 87] and thermal hyperalgesia [88] are controlled in rats upon applying this unique non-viral intrathecal gene transfer method. To examine whether non-viral pDNA-IL-10 gene therapy is effective in other clinically relevant pain problems, allodynia, from a cancer chemotherapeutic animal model using systemic treatment of paclitaxel and mediated by spinal glial IL-1 β , is controlled for greater than 35 days following intrathecal pDNA-IL-10 treatment [72, 78]. A simultaneous increase in transgene IL-10 mRNA with a corresponding decrease in IL-1 β and TNF- α mRNA levels in spinal meninges is also observed. These results suggest that intrathecal pDNA-IL-10 controls pathological pain in paclitaxel-induced allodynia by blocking the lumbosacral spinal actions of IL-1 β and TNF- α . Thus, pathological pain states such as peripheral nerve trauma and chemotherapy-induced

neuropathies may be controlled by intrathecal applications of the gene encoding the anti-inflammatory cytokine, IL-10.

Phagocytosis of non-viral pDNA-IL-10 following Intrathecal Injection

While the underlying mechanisms related to how naked IL-10 transgene gains access to host cell machinery is not well-characterized, several possibilities can be considered. A large literature supports transgene uptake via non-specific phagocytosis by leukocytes [89-94]. Although virtually every cell type is capable of phagocytosis, macrophages are specialized phagocytic cells residing within the healthy and neuropathic meninges [76, 95]. Additionally, spinal cord microglia and astrocytes are ascribed as highly efficient phagocytic cells of the CNS [75, 96]. Phagocytes, like macrophages and glial cells become activated in response to immune stimulation from naked DNA [92, 97, 98] via pattern recognition receptors that identify evolutionarily conserved molecular motifs on pathogens [99]. These pattern recognition receptors span a broad range of receptor families including membrane bound Toll-like receptors (TLRs), for example, TLR9, expressed on phagocytic cells like macrophages and glia [100, 101]. Agonists to TLR9 are currently being explored in clinical trials, and are made up of oligodeoxynucleotide (ODN) cytosine-phosphate-guanine (CpG) stimulatory nucleic acid sequences, which are also present in the pDNA-IL-10 non-viral vector described here for intrathecal gene therapy. CpG-containing ODN's are known to be taken up by cells in a clathrin-dependent manner and bind TLR9 on endosomal vesicles. TLR9 resides in the endoplasmic reticulum before stimulation and is rapidly recruited to lysosomes on activation [102]. In lysosomes of B cells, macrophages, and a subset of dendritic cells [103, 104], TLR9 interacts with internalized CpG pDNA, followed by signal-transmission in a MyD88-adaptor-dependent fashion suggesting resultant proinflammatory consequences. However, it is notable that CpG-pDNA exposure to TLR9-expressing cells do not necessarily result in proinflammatory cytokine production, but rather, can result in anti-inflammatory IL-10 production [105]. Indeed, microglia, also characterized to express TLR9 mRNA and protein, produced significant increases in IL-10 mRNA and protein following exposure to CpG DNA. These studies suggest that intrathecal pDNA-IL-10 is capable of harnessing local immune cells for gene transfer without inducing proinflammatory signaling, and may instead, induce a transient anti-inflammatory milieu that is ultimately beneficial for transgene expression.

Intrathecal pDNA-IL-10 Encapsulated by PLGA Microparticles

While a number of non-viral gene delivery vehicles are being developed to improve efficiency for gene transfer [106, 107], synthetic, biocompatible polymers are promising as they are safe, more flexible in manipulating their chemistry, and simple to manufacture [108]. Although long-duration pain reversal by intrathecal free pDNA encoding IL-10 is demonstrated in animals of pathological pain, several potential limitations exist. These include the dose required to achieve therapeutic benefit (125 ug total in rat), and that two injections are required, a potential limitation for clinical applications. Poly(lactic-co-glycolic acid) (PLGA), approved by the US Food & Drug Administration, has an established history of successful clinical applications for slow release of large molecules, peptides and proteins [109]. PLGA microparticles containing pDNA cargo are readily phagocytosed and can be engineered to additionally microencapsulate factors that enhance transgene uptake and expression for as long as 8 weeks [110], properties that serve as a major advantage for intrathecal therapeutic transgene delivery [111, 112]. These particles are created to microencapsulate pDNA encoding the IL-10 gene. Upon intrathecal injection in neuropathic rats, the PLGA microparticles begin to break down to components of the citric acid cycle, releasing their pDNA-IL-10 contents into the surrounding cellular environment. Indeed, these microparticles were identified, using confocal microscopy, in close association with

cellular nuclei in the subarachnoid meninges close to 2 weeks after the second pDNA-IL-10 injection. These PLGA microparticles co-localize with cells stained for the phagocytic macrophage marker, ED-1 (Figure 1A). This is particularly attractive for spinal pDNA-IL10 gene therapy in future clinical application because pain reversal was observed at doses ~100-fold lower than free plasmid DNA [113], albeit 2 intrathecal injections were required.

However, continued development of PLGA microparticles formulated with pDNA-IL-10 has significantly advanced to achieve enduring suppression of allodynia induced by chronic constriction injury after a single intrathecal injection (~ 10 ug pDNA-IL-10). The morphological characterization of PLGA is spherical with a smooth surface when examined under scanning electron microscopy (Figure 1B). Relief from allodynia in chronic constriction injury-induced neuropathic rats is observed for ~74 days [114]. In the spinal CSF and lumbosacral tissue, significant pDNA-derived IL-10 mRNA increases are observed during full relief from allodynia. These elevations in IL-10 mRNA are absent when allodynia returns, as assessed 93 days after a single intrathecal PLGA-IL-10 injection. Furthermore, in experimental animals treated with a co-injection of empty PLGA microparticles and an equidose of naked pDNA-IL-10, allodynia persists. These results suggest that PLGA microencapsulation of pDNA-IL-10 serves to protect transgene IL-10 breakdown and thereby improves spinal gene transfer. In the same study, this formulation of PLGA microparticles interacts with cells that express the classic monocyte/macrophage marker, MHC II in lumbosacral spinal meninges [114]. Additionally, spinal microglial cells, identified by staining for Cd11b expression using OX-42 antibody IHC procedures, interact with PLGA microparticles [114]. These data are highly promising and continued progress is being made toward minimizing the required pDNA-IL-10 dose for effective pain relief in preclinical studies of animal models [115]. Of the many possibilities currently being explored, long-term pDNA exposure is the most straight forward method to further improve transgene delivery targeted to the intrathecal compartment.

Concluding remarks

Chronic peripheral neuropathic pain, can itself become a disease condition [116], and is a significant national health problem with currently available therapeutics minimally effective. Many of the neuronal and biochemical changes in the spinal cord dorsal horn or the trigeminal/medullary dorsal horn are in part, initiated by and consequences of immune and glial cell signaling [16, 117]. Thus, conditions that activate and maintain sensitization of primary sensory neurons and dorsal spinal cord pain transmission neurons also involve surrounding glial activation. In response to a number of cellular activation pathways that are initiated during neuropathic pain states, active glia, and possibly infiltrating leukocytes, release the proinflammatory IL-1 β and TNF- α that ultimately participate in underlying chronic peripheral neuropathic pain. The anti-inflammatory cytokine, IL-10, is a powerful counter-regulator that controls proinflammatory function. While IL-10 exerts robust suppression of pathological pain induced in a number of animal models of peripheral neuropathy, the potential prophylactic effects of IL-10 to prevent the initiation of pathological pain is largely unknown. This is an important consideration for several clinical pain problems such as chronic post-surgical pain recently characterized in a rat model to involve astrocyte and microglial responses and p38MAPK activity [118, 119]. In people, a surprisingly large percentage (~10-50% depending on procedure) of patients develop persistent postsurgical pain, with a subset of these patients developing severe chronic pain [120]. The application of non-viral pIL-10 gene therapy as a prophylactic treatment for post-operative pain is highly intriguing because the underlying mechanisms resulting in such pain could be prevented, thereby limiting post-surgical complications and additionally minimizing the need for post-surgical opioid analgesic treatment.

Novel and promising gene therapeutic approaches that employ the actions of IL-10 are being developed as therapeutics to treat chronic neuropathic pain conditions. Targeted therapeutic gene delivery to the spinal intrathecal compartment using the biodegradable and biocompatible synthetic polymer, PLGA, that microencapsulates and protects the IL-10, is currently being developed for effective and safe transgene expression. The goal of this work is to develop therapeutic gene delivery methods for clinical utility to treat chronic neuropathic pain.

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Figure 1. PLGA microparticles

(A) The distribution of PLGA microparticles encapsulating pDNA-IL-10 following a single intrathecal injection. For verification that the microparticles could associate with phagocytic immune-cells in the subarachnoid matrix, after a single intrathecal injection of poly-L-glycolide-D-lactide (PLGA), a co-polymer (50:50 MW 75,000) used to microencapsulate a plasmid DNA vector encoding the anti-inflammatory gene, interleukin-10 (IL-10) was conducted. Ten days after subarachnoid (intrathecal) delivery of fluorescently labeled (rhodamine) microparticles encapsulating pDNA-IL-10, animals were deeply anesthetized, and transcardial saline followed by 4% paraformaldehyde perfusion procedures were conducted. Isolated spinal cords were post-fixed and 30 µm cryosections were collected. Spinal cord cross-sections and labeled microparticles (red; rhodamine), cellular nuclei (blue; DAPI), and phagocytic macrophage (green; ED-1) were examined and imaged using confocal microscopy (40X). Microparticles (red) are co-localized with macrophage (white arrow) within the meningeal tissue surrounding spinal cord. PLGA microparticles did not appear in deeper spinal parenchyma.

(B) A representative scanning electron micrograph image of PLGA microparticles encapsulating pDNA-IL-10 that were injected into the intrathecal space and produced enduring reversal from allodynia in rats with unilateral sciatic nerve damage from chronic constriction injury.