Poly(lactic-co-glycolic acid) nanomaterial-based treatment options for pain management: a review

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Neuropathic pain is one of the most intense types of chronic pain; it constitutes a pervasive complaint throughout the public health system. With few effective treatments, it remains a significant challenge. Commercially available drugs for neuropathic pain are still limited and have disappointing efficacy. Therefore, chronic neuropathic pain imposes a tremendous burden on patients' quality of life. Recently, the introduction and application of nanotechnology in multiple fields has accelerated the development of new drugs. This review highlights the application of poly(lactic-co-glycolic acid) nanomaterial-based vehicles for drug delivery and how they improve the therapeutic outcomes for neuropathic pain treatment. Finally, future developments for pain research and effective management are presented.

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Neuropathic pain is a chronic pain syndrome that often occurs after nerve injury or because of a disruption in the nervous system [1,2]. Patients with neuropathic pain often describe their pain to doctors as a shooting or burning [3]. Sometimes, neuropathic pain comes and goes, but it is often chronic and challenging to treat. Neuropathic pain currently has a prevalence of 3–17% with few sufferers achieving adequate pain relief [4]. Nerve damage is often a hallmark in the pathogenesis of neuropathic pain [2,5]. It can occur without any apparent cause, but some of the most common causes of nerve damage include alcoholism, diabetes, chemotherapy, cancer and amputation [6]. Many studies have revealed neuropathic pain-like symptoms induced by these diseases [7–10]. Neuropathic pain is characterized by a hypersensitivity to touch, which is called mechanical allodynia; this makes innocuous stimuli seem excruciatingly painful [11]. In addition to evoked pain, spontaneous pain is also observed in neuropathic pain patients [12]. The symptoms of pain appear to vary from patient to patient but, generally, neuropathic pain is chronic and sometimes severe enough to reduce their quality of life significantly.

The pathogenesis of neuropathic pain has been studied extensively and knowledge on pain has built incrementally. In the early phase, neurotransmitters such as ATP and neuropeptides, which are released from damaged primary afferent neurons, strongly activate glial cells via multiple pathways [13]. In turn, activated glial cells respond by releasing several potentially neurotoxic mediators, including cytokines, chemokines, reactive oxygen species and nitric oxide [14]. These neuroimmune interactions have been widely reported to contribute to the development of neuroinflammation [15]. Immune mechanisms play important roles in the initiation of neuropathic pain and its transition to chronic pain. In the later phase, chronic neuroinflammation-induced synaptic plasticity in the CNS is called central sensitization, which causes widespread pain beyond the nerve injury site [16]. Central sensitization is a driving force for chronic pain, which can allow attacks of pain without provocation.

Although investigations of the mechanisms that drive neuropathic pain broaden our understanding and provide opportunities for finding out therapeutic candidates, neuropathic pain is still refractory to treatment. Even when

Table 1. Available pharmacological treatments for neuropathic pain.

Medication class	Drugs	Recommended stage	Commercial brand
Antidepressants tricyclics	Amitriptyline	First line	Elavil
Serotonin and norepinephrine reuptake inhibitors	Duloxetine, venlafaxine	First line	Cympalta, generic
Anticonvulsants	Pregabalin, gabapentin	First line	Lyrica, Neurontin, Gralise
Topical analgesics	Lidocaine, capsaicin	Second line	Qutenza, Lidoderm
Opioids	Tramadol, oxycodone	Third line	Tramal, Oxycontin
Others	NSAIDs, paracetamol	Limited effect	

NSAID: Nonsteroidal anti-inflammatory drug.

the cause of nerve damage is clear, it is still difficult to prevent the development of pain. Currently, several research initiatives have suggested new therapeutic candidates for treating neuropathic pain. However, few of the novel mechanisms have successfully translated into drugs for clinical use [17]. A brief table of medications used in clinical treatment of neuropathic pain is presented in Table 1.

Unlike acute pain, neuropathic pain does not usually get better with traditional pain killers, such as paracetamol or codeine. Antidepressants and anticonvulsants are often the first line of treatment. The second line is topical analgesics such as lidocaine-capsaicin. The last line of treatment is potent opioids for patients with severe pain who cannot be treated with other drugs. However, these are rarely used in chronic pain patients due to the potential for tolerance and addiction. These drugs mainly act to modulate synaptic plasticity or neuronal excitation [18]. Antidepressants, such as amitriptyline and duloxetine, were initially prescribed for patients with headaches and depression, but are now used regularly for chronic pain [19]. Evidence from previous studies shows that amitriptyline and duloxetine can have an analgesic effect in neuropathic pain in conditions such as diabetes and alcoholism [20,21].

The anticonvulsant gabapentin or pregabalin has also been shown to be safe and effective in pain relief by downregulating the activity of the calcium channel in neurons resulting, therefore, in decreased central sensitization and nociceptive transmission [22]. It seems these drugs are effective because they focus on treating the types of symptoms associated with neuropathic pain. However, clinical surveys show that a small percentage of patients achieve satisfactory pain relief, even with the first-line treatment [4]. Furthermore, most patients do not respond to conventional analgesics. Nowadays, poor management of neuropathic pain impairs patients' quality of life and function. The management of neuropathic pain is challenging and should be the focus of novel approaches to improve the quality of life for patients who have neuropathic pain.

Microglia in neuropathic pain

The previous decade has seen a rapid increase in studies on neuropathic pain pathogenesis, with a unique focus on finding effective therapeutic candidates. Interestingly, there are numerous studies on microglia in pain, showing microglia have emerged as key regulators of pain diseases [23–26]. Microglia are resident macrophages of the CNS and patrol the cellular environment. They are capable of detecting molecular changes and respond to mediators such as neurotransmitters. In response to changes, microglia undergo rapid modifications to their structures and functions. These changes are called microgliosis, which includes proliferation and morphological changes resulting in activation of microglia (Figure 1). In response to various signals, microglia proliferate and enlarge their bodies from ramified to amoeboid shape with shortening processes. This event leads to the production of microglial mediators, which are the hallmark used to evaluate inflammatory condition in the CNS. When activated, they are prone to release pro-inflammatory cytokines and promote the development of inflammation [27].

Microglia can play detrimental or protective roles in the pathogenesis of neuroinflammation and pain. They are considered beneficial when they produce anti-inflammatory mediators, such as IL-4 and IL-10, which can promote tissue repair [29].

Excessive neuroinflammation often involves overactivation of microglia, which leads to increased release and expression of inflammatory microglial mediators in the nervous tissue [30]. Neuropathic pain has been shown to result from chronic neuroinflammation and microglia contribute to this disease via regulation of microglial mediators. Damage-associated molecular patterns, including ATP, high-mobility group box 1 (HMGB1) and pathogen-associated molecular patterns released from damaged neurons can strongly activate microglia after nerve injury [31]. As a result, numerous signaling molecules are altered in microglia and contribute to the development of

Figure 1. Microgliosis and upregulation of multiple signaling in spinal microglia after nerve injury. (A) Microgliosis in the spinal dorsal horn following nerve injury: proliferation and morphological changes as revealed by Iba1 (microglial marker) staining. **(B)** Upregulation of multiple signalling in activated microglia.

DAMP: Damage-associated molecular pattern; PAMPs: Pathogen-associated molecular pattern. Immunostaining images reproduced with permission from [28], licensed with CC BY-NC-ND 4.0.

inflammation. In activated microglia, surface receptors such as purinergic receptors and Toll-like receptors (TLRs), as well as intracellular and secreted signaling molecules such as MAP-kinases and NF-κB, are found in greater abundance [23]. Deletion or inhibition of these signaling factors has been demonstrated to reduce microgliosis and pain behaviors in various animal neuropathic pain models. For example, TLR4-deficiency mice or knockdown of TLR4, TLR2 inhibited nerve injury-induced microglial activation and cytokine expression [32,33]. Spinal blockade of P2X4R signaling reduced mechanical allodynia, a cardinal feature of neuropathic pain [34]. Notably, NF-κB and p38 MAPK are two major pathways that participate in most of these regulations in microglia in response to microglial activators [35–37]. The p38 group is one of the four main sub-groups of MAP kinases, a family of evolutionally conserved molecules that play a critical role in cell signaling and gene expression.

It has been well documented that nerve injury strongly induces upregulation of the chemokines MCP-1, CCL2 and neuronal proteases such as metalloproteinase-9 and caspase-6 in primary sensory neurons [38]. These molecules released from the damaged dorsal root ganglion (DRG) neurons contribute to neuropathic pain via CCR2 receptor, which has been implicated in activated microglia. Besides, CX3CL1 is primarily produced in the DRG and dorsal horn neurons and further upregulates CX3CR1 receptor expression in spinal microglia after nerve injury. These signaling molecules are activators of p38 MAPK through activating upstream kinases, such as MAP2K3 and MAP2K6. Numerous downstream kinases are further activated, including members of the MAPK-activated protein kinase family, MAPK-interacting kinase and mitogen- and stress-activated kinase. Additionally, phosphorylated p38 MAPK (p-p38 MAPK) translocates to the nucleus and phosphorylates various transcription factors, including cAMP response element-binding protein, thus activating transcription. Prolonged activation

of the p38 MAPK pathway induces the expression of various genes, including pro-inflammatory cytokines [39]. Similarly, the NF- κ B pathway plays a crucial role in regulating the inflammatory response in microglia. Stimulation of a wide variety of cell-surface receptors, such as TLR2 and TLR4, purinergic receptors P2X4R and P2X7R and chemokine receptors CX3CR1 and CCR2 lead to NF- κ B activation and fairly rapid changes in gene expression. The primary mechanism for canonical NF- κ B activation is the inducible degradation of I κ B α , one of the two NF- κ B inhibitors triggered through its site-specific phosphorylation by a multisubunit I κ B kinase (IKK) complex. These events result in rapid and transient nuclear translocation of canonical NF- κ B members, predominantly the p50/RelA and p50/c-Rel dimers, which subsequently initiate the transcription of primary response genes such as TNF- α , IL-6 and IL-1 β [40]. Many pain researchers have reported that inhibition of these pathways in microglia can effectively improve neuroinflammation and pain [41,42]. Increasing evidence from pain studies, which focus on how microglia affect synaptic plasticity in the spinal cord and brain, has suggested the central role of microglia in the pathogenesis of pain diseases.

Recent strategies to study microglia functions & pain management

A limitation in microglia research is the lack of effective and selective tools. Recently, some novel tools have been developed to overcome this limitation. Among these, chemogenetics and optogenetics have received significant attention for gaining selective control in teasing out the roles of neurons and glia in neurological diseases. These novel techniques have shown their advantages and further supported the crucial roles of microglia in the induction of pain.

The ability to manipulate genetically defined cells using engineered receptors has been entertained by pain researchers as becoming more than a research tool. A form of chemogenetics that involves the expression and activation of engineered G-protein coupled receptors (GPCR) has been widely employed in pain studies. Designer receptors exclusively activated by designer drugs (DREADDs), which can modulate transgenic receptors using artificial pharmacologic agents, are the most plausible tool [43,44]. DREADDs are designed to be activated or inhibited based only on the binding of synthetic chemicals, not by endogenous GPCR ligands. Mechanisms of action of DREADDs are similar to other endogenous GPCRs, except that they are controlled only by chemical ligands [45]. This allows scientists to study the functions of selectively targeted cell types. In terms of neurological diseases, some previous studies have employed this method to discover a neuron's functions as part of a pain circuit [46,47]. For instance, inhibition of sodium channel Nav1.8 in the DRG sensory neurons by DREADDs has been shown to suppress neuronal activity, thereby reducing mechanical allodynia in a mouse osteoarthritis model [48]. Recently, it has become clear that microglia are crucial players in the pathogenesis of neuroinflammation and pain and pain researchers have made efforts to control microglia activation by DREADDs. For example, DREADD-expressing microglia have demonstrated the role of microglia in pain, suggesting DREADDs as promising tools for microglia study, as well as the promise of this novel approach for pain relief [43].

In addition to DREADDs, optogenetics refers to a set of techniques, in which light-sensitive proteins can be activated by a particular light frequency stimulus [49]. These proteins, called opsins, are introduced into specific cell populations, allowing selective stimulation of targeted cell types with fiber optics to deliver light to the cells. Depending on the properties of the opsins selected, optogenetics can be used to inhibit or stimulate cell activity. Channelrhodopsin (ChR) is engineered to be selective for cation entry and activated by blue light to elicit activation of cells. Halorhodopsin (NpHR), on the other hand, is activated by yellow light and promotes inhibition by selective anion entry. In terms of pain sensation, optogenetics has been applied early in the studies on neuronal activity. Nav1.8 positive primary afferent neurons were selectively targeted to express ChR2 channels. These neurons were shown to elicit nociceptive behaviors in response to blue light stimulation [50]. Another study also achieved a similar result with Nav1.8–ChR2⁺ spinal dorsal horn neurons in mice [51]. In contrast, inhibitory NpHR expression in sensory nociceptive neurons reduced pain hypersensitivity and inflammatory condition when activated [52]. In another case, optogenetic activation of inhibitory neurons to evoke GABA release was sufficient to prevent pain behaviors, suggesting its therapeutic potential [53]. However, the application of optogenetics on glial cell populations is still limited. There have been several reports showing success in optogenetic manipulation of astrocytes [54]. For astrocyte-specific opsin expression, some studies used Cre/loxP or tetO-tTA systems to generate astrocyte-ChR2 transgenic mice that express ChR2 in astrocytes at quantities that allow optogenetic astrocyte manipulation [55,56]. Unlike astrocytes, optogenetic application on microglia has been poorly studied so far. There are some concerns that opsin activation may not be able to modulate microglia activation. The possibility of optogenetic manipulation in microglia has proven tantalizing, but challenging, for pain researchers. However, a nanomaterial-based delivery

method, which has shown its effect on enhancing microglia targeting, may create opportunities to overcome these obstacles.

Nanomedicine for neuropathic pain management & poly(lactic-co-glycolic acid) copolymer

Over the last decade, nanotechnology, a field of study in which the subject must have at least one dimension in the nanometer scale (<100 nm), has been developed, introduced and applied in multiple fields. Nanomedicine has become a thriving research topic, applying nanomaterials to unmet medical needs. These medical purposes include research, diagnosis, prevention and treatment of various diseases. The development of nanomaterials and their clinical applications in medicine have ushered in a new era for drug delivery systems. Nanoparticles can improve the therapeutic efficacy of the loaded drugs. Nanomaterial-based carriers are designed to overcome biological barriers, which can limit effective drug delivery [57]. For basic drug administration methods such as the oral route, injection and nasal route, the efficiency of drug delivery transport can be affected by the blood-brain barrier, nasal condition, skin, the oral mucosa or the small intestine [58]. These natural barriers prevent foreign materials from entering but do allow small specific molecules to cross. These barriers have become a major challenge for the pharmaceutical industry. This effort has been ongoing and has resulted in a number of applications and products, with nanomaterials becoming available in both the research and pharmaceutical markets recently [59].

Nanomaterials, with their unique physicochemical properties, open up a new strategy for drug development. These novel properties include small size, particle size distribution and enhanced surface energy, making nanoparticles more active [60]. Also, nanomaterials can be designed and engineered to achieve different properties with regard to size, shape, surface, chemical composition or intrinsic structure, to optimize their uses [61]. A careful and optimal design for nanoparticles can lead to alteration in pharmacokinetics, including absorption, distribution, metabolism and elimination, making it possible for them to achieve a specific biological outcome. For these reasons, nanomaterials have been recognized as potential and innovative medical solutions in medical practice and research.

Over the last few years, scientific publications on nanomaterials and their applications in various diseases have rapidly increased. After undergoing the process of approval, a set of nanomedicines have been successfully introduced to the pharmaceutical market [62]. These nano-drugs can be produced in the form of a powder, solution, soft capsule or suspension and are now commercially available for patients with atopic dermatitis, iron deficiency and fungal infection, particularly for patients with cancers [63–66]. This development shows the maturation of nanomedicine as a research area. In neuropathic pain, there are now only three nanomedicines that have been approved for clinical use by the US FDA and indicated for pain treatment [67]. They are triamcinolone acetonide extended-release (ER) injectable suspension, morphine sulfate and liposomal morphine sulfate. Pain nanomedicine has been emerging as an innovative solution in response to the current needs of effective treatment. However, available treatment options are still weak. This weakness is both a challenge and an opportunity for pain research to learn from nanomedicine. To date, some pain studies have employed nanomaterial-based carriers to deliver higher amounts of drugs to desired targets, to promote therapeutic efficacy and reduce unwanted side effects. For these purposes, several types of nanomaterials have been used, such as stable lipid nanoparticles, gold nanoparticles, poly(anhydride-ester) and, in particular, poly(lactic-co-glycolic acid) (PLGA) copolymer. Applications of PLGA copolymer make up a large proportion of the total studies on pain nanomedicine. Given the significant contributions of this polymer to the field of neuropathic pain treatment, in the present review, we cover an introduction of PLGA copolymer and offer a unique focus on the application and development of pain nanomedicine with PLGA nanoparticles. Also, several achievements that have been obtained with other nanomaterials are also presented in the following sections.

PLGA copolymer is one of the most commonly used biodegradable synthetic polymers in medicine [68]. Polyglycolic acid (PGA) is a hydrophilic and highly crystalline polymer. In contrast, the physical, chemical and mechanical properties of polylactic acid (PLA) can be flexible due to the presence of a pendant methyl on the α carbon. Endogenous pathways can easily remove both PLA and PGA once they are naturally degraded inside the body [69]. These safe chemical properties enable PLA and PGA to be used in various biomedical products and devices, which have been approved by the FDA. The copolymer PLGA, which consists of PLA and PGA (chemical structure of PLGA and its monomers are shown in Figure 2), is now one of the most defined nanomaterials available for drug delivery. In particular, the design and formation of PLGA nanoparticles can be performed at different ratios between its constituent monomers, PLA and PGA, to achieve the desired dosage and timed release of encapsulated drugs [68,70]. Due to these excellent properties, PLGA has been among the most attractive polymeric candidates and the application of PLGA has shown immense potential as a drug delivery carrier.

There are numerous excellent reviews that focus on nanomaterials and their applications in several diseases [71–76].

Figure 2. Chemical structure and morphology of poly(lactic-co-glycolic acid) nanoparticles.

PGA: Polyglycolic acid; PLA: Polylactic acid; PLGA: Poly(lactic-co-glycolic) acid.

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Table 2. Current nanomedical options for neuropathic pain treatment.

Pain model	Active ingredient	Carrier	Administration method	Pain-reduction time	Ref.
(Rat) SNL-induced NP	p38 siRNAs	PLGA nanoparticles	Intrathecal injection	7 days	[77]
(Rat) thermal hyperalgesia and capsaicin-induced pain	TRP siRNAs	Solid lipid nanoparticles	Topical and intradermal application	Enhanced	[78]
(Mouse) MIA-induced osteoarthritis	p47phox siRNAs	PLGA nanoparticles	Intra-articular injection	>2 weeks	[79]
(Mouse) MIA-induced osteoarthritis	p66shc siRNAs	PLGA nanoparticles	Intra-articular injection	>2 weeks	[80]
(Rat) SNL-induced NP	miRNA 146a-5p	PLGA nanoparticles	Intrathecal injection	11 days	[28]
(Rat) CCI-induced NP	TNF siRNAs	gold nanoparticles	Intra-hippocampal injection	6 days	[81]
(Rat) CCI-induced NP	IL-10 plasmids	PLGA microparticles	Intrathecal injection	>2 weeks	[82]
(Rat) SNL-induced NP	foxp3 plasmids	PLGA nanoparticles	Intrathecal injection	7 days	[83]
(Mouse) partial sciatic nerve injury-induced NP	Lamotrigine	PLGA nanoparticles	Intravenous injection	48 h	[84]
(Mouse) radiant mouse tail-flick model	Capsaicin	Nano-lipoidal biocompatible topical carrier	Topical application	Enhanced	[85]
(Mouse) chronic compression of the dorsal root ganglion	Bupivacaine	PLGA nanoparticles	Unilateral injection	Enhanced	[86]
(Mouse) spared nerve injury-induced NP	Morphine	Poly(anhydride-ester) backbone	Intraperitoneal injection	24 h	[87]
(Mouse) mouse model of acute nociception	Opioids	PLGA nanoparticles	Subcutaneous administration	6 days	[88]
(Rat) CCI-induced NP	Cannabinoid CB13	PLGA nanoparticles	Oral administration	11 days	[89]
(Rat) STZ-induced diabetic Peripheral neuropathy.	Curcumin	Poly(PEGMA-DMAEMA-MAO) polymer	Sublingual intravenous injection	>2 weeks	[90]
(Rat) sciatic nerve ligation-induced NP	Curcumin	PLGA nanoparticles	Intravenous and Intrathecal injection	Enhanced	[91]

CCI: Chronic constriction injury; MIA: Mono-iodoacetate; NP: Neuropathic pain; PLGA: Poly(lactic-co-glycolic acid); SNL: Spinal nerve ligation; STZ: Streptozocin.

In this review, different applications of treatments based on nanomaterials (a summary is presented in Table 2) for neuropathic pain treatment will be described, suggesting promising solutions as a base for future research and clinical practice in the field of neuropathic pain. Various types of drug-encapsulated nanoparticles have shown their significant effects on reducing pain behavior by sustained drug release and, particularly, by enhancing microglia targeting. In addition to reviewing the previous achievements, we further discuss future prospects with regard to

developing this novel method in combination with other materials, such as DREADDs, as well as optogenetics for microglia study and the therapeutic potential of these approaches for neuropathic pain management. The comprehensive view we offer in this review may identify opportunities for the development of nanomedicines with PLGA nanoparticles in the field of neuropathic pain, which is still refractory to treatment.

Gene therapy combined nanomaterial-based delivery systems

Precision medicine and gene therapy have shown great potential for the treatment of diseases that are challenging to treat [92–95]. However, this potential predominantly exists in research laboratories and its application is still experimental. Gene therapy is an experimental technique, in which the gene of interest is replaced, inactivated or silenced or a new gene is introduced into the cell to discover new gene functions [96]. This has become possible through the advances of genetics and bioengineering that enable manipulation of vectors for the delivery of genes or the introduction of multiple gene editors including synthetic RNAi, miRNA and long non-coding RNA (lncRNA), in addition to plasmid or CRISPR/Cas9 for gene silencing, enhancing or editing [97]. This therapy has shown great potential for precision medicine since it promises to treat certain diseases at their origins.

In the field of neuropathic pain, gene therapy with its unique advantages seems to be an attractive modality for the treatment of this chronic syndrome [98–102]. The introduction of specific genes into neurons has helped in making significant progress in understanding the causation of central sensitization [103–106]. Recently, achievements in the application of gene therapy have offered the potential to target the modulatory role of microglia in neuropathic pain [107–109]. For example, a knockout of the p38 pathway in spinal microglia suppressed the development of mechanical allodynia in various neuropathic pain models [37,110]. However, as mentioned previously, a critical issue in microglia research is the lack of selective tools. Although some efforts have been made to achieve selective deletion or over-expression of the targeted genes in microglia, there are still some limitations with currently available gene delivery methods [111]. Notably, pain researchers have tried different techniques for gene targeting in microglia using non-viral and viral methods *in vitro* for eventual application to the *in vivo* setting. However, as transduction of genes of the microglia is rare, none of these methods have shown reliable results. Recombinant viruses such as lentiviruses and adeno-associated viruses have been successfully used to target various cell types in the brain, but have been shown to be inefficient for targeting immune cells such as microglia [112]. Our research team has developed a promising nonviral nanomaterial-based vehicle for combination with gene therapy. This method has shown great potential for microglia targeting, thereby enhancing gene delivery to microglia and promoting therapeutic effects of gene therapy specifically in these glial cells.

siRNA-encapsulated nanoparticles

siRNA is one of the most important advances in biology for regulating the expression of genes [113–115]. Due to its ability to inhibit specific genes, siRNAs can be used as a tool to study single gene functions. Notably, in microglia study, upregulation of multiple signals after nerve injury can be well defined using siRNAs to silence genes of interest specifically. For this purpose, the p38 pathway is a concise and obvious target since this is one of the most active inflammatory pathways found in activated microglia. Pharmacological inhibition of p38 MAPK activity or knockout of p38 have shown effects on reducing pain in rodent models of neuropathic pain [116]. In our *in vitro* experiments, in which cultured microglia were transfected with p38 siRNAs to promote overexpression, lipopolysaccharides (LPS)-induced p38 activation was effectively suppressed [77]. The next step was the introduction of siRNAs *in vivo*, which requires ‘carriers’ that can deliver p38 siRNAs specifically to spinal microglia to promote an analgesic effect. As a carrier PLGA nanomaterials were employed that exhibited an excellent performance. The p38 siRNA-loaded PLGA nanoparticles were mainly uptaken by spinal microglia, not by neurons or astrocytes, after intrathecal injection [77]. The unique characteristics of microglia may explain this. They are the macrophages of the CNS, allowing them to take up nanoparticles by phagocytosis, which other nervous cell types cannot do [117]. The stable and slow release of siRNA within microglia brought about a significant effect on repressing p38 pathway activation, subsequently alleviating mechanical allodynia in the neuropathic pain-induced rats for 7 days [77]. Consistently, targeting microglia by p66shc siRNA-encapsulated PLGA nanoparticles has also shown excellent analgesic effect for 9 days by one intrathecal injection [UNPUBLISHED DATA]. Also, in another model of neuropathic pain by mono-iodoacetate-induced osteoarthritis, p66shc siRNA and p47phox siRNA nanoparticles were both observed to suppress the production of oxidative stress and mitochondrial dysfunction for over 2 weeks after injection [79,80]. These studies are strong evidence that siRNA loaded nanoparticles can achieve excellent analgesic effects, compared with using original drugs. Furthermore, this suggests that PLGA nanoparticles are an

effective carrier for sustained siRNA delivery to the spinal microglia. The release profiles of siRNA from PLGA nanoparticles were carried out in each study to demonstrate the stability of siRNA release in a sustained manner. The release profiles revealed a mechanism for long-term effects of siRNAs on repressing specific inflammatory gene expression in spinal microglia. Multiple signals are expressed and upregulated once microglia are activated after nerve injury. Using siRNAs to block the expression of targeted genes, specifically, allows scientists not only to study the functions of single genes but also to uncover the therapeutic potential of its specific siRNA. The PLGA nanoparticle represents a promising tool that holds the potential to enhance microglia targeting by siRNAs due to its ability to protect siRNAs from inactivation and improve solubility and bioavailability.

In addition to targeting gene expression in microglia to treat neuropathic pain in a rat model, a previous study has revealed TRP siRNA-loaded solid lipid nanoparticles reduced pain sensation via effective silencing of skin TRPV1 following topical and intradermal application, while uncoated TRP siRNA did not show any effect. This suggests a solid lipid nanoparticle-based carrier as a novel method to deliver siRNA across the skin barrier [78]. In addition, another work has shown that TNF-siRNA complexed to gold nanoparticles and injected into the hippocampus effectively silenced TNF gene expression for up to 11 days, thus delaying the onset of mechanical allodynia in chronic constriction injury-induced rats. Therapeutic complexes with nanoparticles led to the development of a safe and long-lasting silencing effect of siRNA on TNF gene expression [81]. These results indicate that nanomaterial-based carriers can greatly enhance transfection efficiency of siRNA in *in vivo* testing.

miRNA-encapsulated PLGA nanoparticles

miRNA and siRNA are small noncoding RNAs that share many similarities [118,119]. Both are short duplex RNA molecules that exert gene silencing. The molecular mechanisms of action of these small RNAs are rather similar. The main difference between siRNAs and miRNAs is that siRNAs are highly specific with only one mRNA target, whereas miRNAs can have multiple targets [118]. Therefore, each RNA holds its own advantages in research and therapeutic approaches. In the field of neuropathic pain, many miRNAs have been identified as participating in the regulation of neuroinflammatory processes in nervous tissue [120–122]. These miRNAs, such as miRNA 146a, miRNA 146b, miRNA 155, miRNA 30 are master post-transcriptional regulators that hold the potential power to modulate the immune and inflammatory responses negatively [122–124]. Among these, miRNA 146a-5p is one of the top candidates that has been found to target multiple inflammatory pathways. Several studies have reported that miRNA 146a-5p attenuated neuropathic pain behaviors in various models [123,124]. However, most of these effects only lasted for a short time. Interestingly, when miRNA 146a-5p was encapsulated into PLGA nanoparticles and then intrathecally administered to the spinal cord of neuropathic pain-induced rats, a long-term pain relief was obtained, which lasted for 11 days postinjection [28]. miRNA nanoparticles showed significant effects on suppressing multiple adaptor protein expression, thereby inhibiting multiple inflammatory pathways. These events were promoted and maintained in the spinal cord by the slow release of miRNA from nanoparticles. Given the power to modulate inflammatory response, similar to miRNA 146a-5p, miRNA 155, miRNA 30 are expected to achieve similar analgesic effects when encapsulated with PLGA nanoparticle carriers. However, these expectations require further investigations.

Plasmid vector-encapsulated PLGA nanoparticles

In addition to small noncoding RNAs (miRNAs, siRNAs), which work for gene silencing, the introduction of a new gene or overexpression of a gene of interest is broadly applied to the study of gene function. To do this, vectors, the gene-delivery systems, can be used [125]. The two primary gene therapy systems available today are viral and nonviral vectors [126,127]. Nonviral systems comprise all physical and chemical systems that do not use viruses; one example is plasmid vectors. In contrast to pro-inflammatory genes, several anti-inflammatory genes have been demonstrated to contribute to the resolution of inflammation and pain [128–130]. To test the protective roles of these genes, some pain researchers have made efforts to introduce genes to glial cells for overexpression. For example, a study reported that intrathecal injection of plasmid DNA encoding IL-10 produced prolonged reversal of neuropathic pain [131]. However, they only showed the transient effects of the transfection of these beneficial genes on reducing pain. Nanomaterial-based carrier systems can overcome this limitation. This is shown by the intrathecal injection of plasmid DNA-encoding IL-10-encapsulated PLGA microparticles, which promoted a long-term reversal of neuropathic pain in a rat model [82]. Consistently, our *in vivo* experiments, in which the rats with neuropathic pain were treated with intrathecal injection of foxp3 plasmid-loaded PLGA nanoparticles, showed pain relief for 7 days with a single injection [83]. In that study, the anti-inflammatory effects of foxp3 plasmids were

prolonged by PLGA nanoparticles, which promoted stable transfection. This result strongly suggests that we can enhance the efficacy of gene transfection by plasmids or transduction by virus vectors through the entrapment of these vectors with nanomaterial-based carriers. Notably, this novel method has shown its potential in promoting microglia targeting, which is a hallmark for treating neuropathic pain.

Drug-encapsulated nanomaterial-based carriers

In the previous section, we discuss the commercially available drugs, that are clinically prescribed for neuropathic pain patients. First-line treatments include the tricyclic antidepressants such as amitriptyline, the selective serotonin and noradrenaline reuptake inhibitors such as duloxetine and calcium channel $\alpha_2\delta$ -ligands such as gabapentin. However, treatment efficiency is still limited, with only a small percentage of patients achieving partial pain relief. Also, the side effects associated with these drugs may limit their uses in neuropathic pain patients [132]. Some concerns have arisen, such as the relatively nonspecific mode of action of amitriptyline, which may cause a broad range of adverse effects. Also, pregabalin administration can promote somnolence. Many studies have reviewed the analgesic effects of these drugs as well as their unwanted side effects on neuropathic pain symptoms in patients with diabetes [133,134]. In this review, we provide evidence indicating a possible solution to support the therapeutic effects and limit the adverse effects of these drugs. The impacts of these drugs are aimed at targeting recycling mechanisms of neurotransmitters in neurons. However, some drugs, such as gabapentin, amitriptyline and duloxetine, can affect microglia activation [135–137]. When being transported by PLGA nanoparticles, the encapsulated drugs were mainly uptaken by the spinal microglia, not by neurons or astrocytes, after intrathecal injection, promoting higher concentrations of selected drugs to be delivered to the microglia [77]. This method suggests that with low doses of drugs, enhanced microglia targeting by PLGA nanoparticle carriers can provide analgesic efficacy and the low dose would limit the side effects caused by the high doses. We initially compared the effects of several drugs between using drugs alone and loading with PLGA nanoparticles. In the former case, the analgesic effects lasted only several hours and disappeared entirely within 1 day after a single intrathecal injection. Interestingly, at the same time, the rats that were injected with drugs encapsulated in PLGA nanoparticles showed pain relief for 7 and 9 days, respectively [UNPUBLISHED DATA]. These results highlight PLGA nanoparticles as promising tools for drug delivery, to promote long-term analgesic efficiency. By using this method, drugs can be transported then released in a sustained manner, thus extending the biological half-life. Moreover, enhanced spinal microglia targeting by intrathecal injection of nanoparticles may limit the side effects of these drugs on the brain, which are usually observed with oral administration.

An investigation by Lalani *et al.* aimed to develop the anticonvulsant lamotrigine encapsulated in PLGA nanoparticles to deliver higher amounts of the drug to the brain. The nanoparticles would improve the biodistribution and pharmacokinetic profile of the drug allowing a prolonged duration of analgesia and a lower chance of side effects following intravenous administration [84]. Capsaicin, a drug for the topical treatment of neuropathic pain, is now considered the second-line treatment. Poor patient compliance is often associated with several side effects like irritation, burning sensation and erythema. To enhance the analgesic effect and decrease skin irritation, a study has developed capsaicin-loaded nanolipoidal biocompatible topical carriers. The analgesic effect was observed to be enhanced more substantially than that of the conventional cream while the most alarming problems of skin-irritation and redness were successfully controlled [85]. In addition to capsaicin, lidocaine and bupivacaine are also indicated as the second-line treatment. It has been demonstrated that the slow, sustained delivery of bupivacaine by PLGA nanoparticles, when injected unilaterally into the L3 and L4 DRGs of mice, may prevent the occurrence of neuronal hyperexcitability, which could develop into chronic neuropathic pain [86].

It is worth noting that opioids, the third line treatment for neuropathic pain, are strong pain-killers, but are only prescribed when patients do not respond to the first and second-line treatments. Moreover, their relatively short half-life of approximately 4 h often necessitates multiple administrations per day. Although their antinociceptive effects are very effective for pain management, severe abuse and overdose potential associated with repeated opioid administration are an important problem that limits their use. To address this, Lax *et al.* have developed a new polymer form of morphine known as PolyMorphine, which has monomeric units of morphine incorporated into a poly(anhydride-ester) backbone [87]. This polymer showed its effects on blocking spared nerve injury-induced mechanical hypersensitivity in mice for 24 h postadministration, longer than the analgesic effect of free morphine, which lasted for 2 h. Notably, a 2017 study by Kovaliov *et al.* has reported the formulation of fentanyl-bearing PLA and PLGA nanoparticles, which showed their success in delivering therapeutically relevant doses for 6 days in a mouse pain model after a single subcutaneous dose [88]. These studies suggest that the sustained release of opioids

from nanoparticles provides prolonged pain relief while avoiding multiple dosing, thus reducing the potential of abuse and overdose.

Recently, exogenous cannabinoids have been demonstrated to be effective in a range of experimental neuropathic pain models [138,139]. A previous study has revealed PLGA nanoparticles containing synthetic cannabinoid CB13 showed sustained pain-relieving effects, lasting up to 11 days after one oral dose [89]. Another work further developed CB13-loaded lipid nanoparticle formulations through solvent-emulsion evaporation. It optimized them in terms of physicochemical properties, long-term stability and integrity under simulated gastric conditions for oral delivery of CB13 [140]. Notably, in diabetic neuropathy, curcumin extracted from the natural medicine turmeric rhizome has been reported to ameliorate diabetic neuropathic pain due to its anti-inflammatory and antioxidant properties [141]. However, it has poor metabolic stability *in vivo* and low bioavailability. To overcome this, a study used amphiphilic polymer macromolecule (PEGMA-DMAEMA-MAO)-loaded curcumin to improve its targeting and bioavailability. The results showed that the nanoparticle-encapsulated curcumin treatment decreased the up-regulation of the P2Y12 receptor in the DRG and decreased mechanical and thermal hyperalgesia in diabetes mellitus rats [90]. Also, another paper has demonstrated the antinociceptive effects of curcumin-loaded PLGA nanoparticles in various models of pain: formalin test, zymosan-induced hyperalgesia and sciatic nerve ligation-induced neuropathic allodynia and hyperalgesia [91]. These results support the concept that nanomaterials hold great potential as novel carriers that can deliver drugs to desired targets. This method can be done in a sustained manner, thus improving their bioavailability, promoting therapeutic efficacy in treating neuropathic pain and reducing unwanted side effects.

Future perspective

The advent of nanomaterial-based vehicles represents a significant advance in the field of drug delivery for treating neuropathic pain. The current options for nanoparticle design, for encapsulating drugs, provide potential applications for pain management in research and an opportunity for further clinical trials. PLGA nanoparticles have shown advantages by enhancing spinal microglia targeting and sustained drug release. The successful applications of gene therapy or drug-encapsulated nanoparticles to treat neuropathic pain confirmed the broad potential of this promising delivery system. However, challenges always exist together with opportunities. The biggest challenge that nanomedicine faces at present is meeting all the safety requirements for clinical use approval. Most of the reports regarding the safety and efficacy available to us today are from animal studies, mostly in mice or rats. None of the studies have been conducted to assess these effects of nanomedical therapeutic candidates in human with chronic neuropathic pain. There are still multiple challenges regarding the assessment of pain nanomedicine in clinical trials toward the introduction of it in clinical practice. The structure and function, as well as their characteristics, composition and interactions with biological systems, of these therapeutic candidates are among the most significant concerns. If the biocompatibility of the nanomedical products cannot be established then their application may raise safety concerns. PLGA nanoparticles represent unique advantages, as compared with other polymers, due to their biocompatibility, tailored release rate, potential to modify properties for better delivery efficacy and, particularly, approval for clinical use in humans by the FDA [142]. It is essential to identify the properties and to understand the mechanisms by which the active ingredients engineered within nanomaterials interact with living systems, to understand the hazards or possible risks. Further studies are needed to assess how nanomedical treatments penetrate cells and tissues and the respective biological distribution, degradation and excretion.

The second issue is the efficacy of drug delivery to the desired target, which is associated with entrapment efficiency and selective targeting. While most of the applications were performed with hydrophilic drugs, an improvement over nonencapsulated drugs is a separate challenge. The design of nanoparticles can be modified to improve the solubility of the hydrophobic drugs, thus improving their bioavailability *in vivo* and allowing more effective drug delivery. For example, modifications of surface charge, size, nanoparticle shape and stealth properties are among the most critical to enhance encapsulated reagents most effectively. The use of the emulsification solvent evaporation technique, the most common method used for the preparation of solid polymeric nanoparticles, may help with the encapsulation of hydrophobic drugs [143]. Another possibility is the use of the phase separation (coacervation) technique in the nanoparticle preparation process [144]. This process allows solutions to be prepared by mixing polymers and solvents in appropriate ratios, thus enabling a combination of hydrophilic and hydrophobic drug encapsulation. This technique holds the potential to combine multiple drugs in a single formation of nanoparticles, which may enhance therapeutic efficacy through multiple targeting. Notably, a recent study by Herneisey *et al.* has presented a quality by design approach used to design pain nanomedicine [145]. They applied a unique combination

approach that compared multiple linear regression and boosted decision tree regression, using a partial least squares regression method. This approach can enable them to determine which steps in the nano-emulsification processes control nano-emulsion droplet diameter, stability and drug loading efficacy. Enhanced quality product and process understanding by a quality by design approach may result in higher-quality products for more effective uses in *in vivo* testing. Although PLGA nanoparticles have demonstrated their unique advantages in enhancing microglia targeting, it is not enough to guarantee selectivity over nonmicroglial cells. Encapsulating the highly specific markers of microglia in addition to drugs in a formation of a PLGA nanoparticle may help promote targeted treatment to work and allow absolute targeting without altering other essential processes. All these efforts can be made to obtain an optimal design for nanoparticles that best suits the selected drugs. Once the optimal formulation is obtained, some limitations can be overcome to gain the most effective drug delivery, thus promoting therapeutic efficiency.

Despite these challenges, nanomedicine is still making beneficial progress as many clinicians and researchers are currently tackling these difficulties. For long-term study, valuable data on potential applications as therapeutic candidates will come from animal studies. Basic research has made significant strides toward our understanding of the mechanisms that drive neuropathic pain, such as the central role of microglia. Combination treatments that specifically target microglia and nanomaterials should be considered as holding therapeutic potential for effective neuropathic pain management. For better insights into the microglial regulatory framework in pain regulation, new and specific tools such as DREADDs and optogenetics should allow for specific targeting of microglia above other nervous cell types. With the paucity of specific regulators for microglia, these novel techniques will also allow for better insights into the roles of microglia in various neuroinflammatory and pain states. Also, the applications of flow cytometry and RNA sequencing profiles in various models of pain on microglia provide valuable resources for further investigations of new therapeutic candidates. Some new players have been emerging in the mechanisms of inflammatory and neurological diseases, such as long noncoding RNAs, peptides and proteins. Combination of these therapeutic strategies with nanomaterial-based delivery systems will be the subject of further development in the field.

Conclusion

Neuropathic pain is a chronic syndrome that continues to influence the lives of a high percentage of patients negatively. Overcoming the challenges in effective pain management may seem like a herculean effort, but it is possible. This article provides a review of the enormous potential of PLGA nanoparticles for drug delivery in treating neuropathic pain. The recent advances in nanomedical options include siRNA, miRNA, plasmid vector or drug-encapsulated PLGA nanoparticles, which have shown significant analgesic effects. The list of potential applications continues to grow because many possible nanomedical options have now been developed and refined. Beyond what we have outlined in this review, further research would be required for the broader application of nanomedicine in pain management. We believe the introduction of nanomedicine for pain management will soon become available clinically.

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Executive summary

Introduction of neuropathic pain & available treatment

- Neuropathic pain characteristics and underlying mechanisms are introduced. Commercial drugs that are currently available for patients with neuropathic pain are presented.

Microglia in neuropathic pain

- Microglia, the resident macrophages in the central nervous system, play modulatory roles in the pathogenesis of neuropathic pain.

Recent strategies to study microglia functions & pain management

- Emerging strategies to study microglial function and effective pain management in response to the needs of effective and selective tools are presented.

Nanomedicine for neuropathic pain management & poly(lactic-co-glycolic acid) copolymer

- The development of nanomedicine and nanomedical options for neuropathic pain treatment is introduced. Poly(lactic-co-glycolic acid) nanoparticles represent promising delivery carriers in the field due to their unique properties and outstanding advantages.
- Applications of nanomaterial-based delivery systems in combination with other methods, including gene therapy (siRNAs, miRNAs, plasmid vectors) and conventional drugs are reviewed.

Future perspective

- Challenges that pain nanomedicine faces and future perspectives to overcome obstacles and to develop practical tools for pain management are discussed.

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