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Perspective

Contribution of Small Extracellular Vesicles from Schwann Cells and Satellite Glial Cells to Pain Processing

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Abstract: Extracellular vesicles (EVs), including exosomes and microvesicles, are membrane-bound particles released by cells into extracellular space. These vesicles carry various molecules, such as proteins and lipids, and can serve as mediators of intercellular communication. EVs have been implicated in the communication between different cell types in the nervous system, for instance, the neurons and glial cells of the central nervous system (CNS) and peripheral nervous system (PNS). Satellite glial cells (SGCs) surround and support neurons in the sensory ganglia of the PNS, and it has been proposed that the EVs released by SGCs may contribute to the processing of pain-related signals and features. This includes the modulation of neuronal activity, the release of pro-inflammatory signaling molecules, and sensitization. A noticeable finding is that EVs can transfer bioactive molecules, including proteins and microRNAs (miRNAs), between cells, influencing cellular functions such as gene expression regulation involved in the transmission and modulation of pain signals. Schwann cells (SCs) also release EVs. SC-derived EVs sequester TNFR1, influencing TNFα activity and regulating neuroinflammation in peripheral nerve injuries. Understanding peripheral glia's EVs role in pain processing is an emerging area in neuroscience. Here, the latest findings, challenges, and potential are presented to encourage future research.

Keywords: satellite glial cells; extracellular vesicles; pain; Schwann cells; glia

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1. Introduction

Extracellular vesicles (EVs) are recognized as crucial components facilitating cell–cell communication [1], especially in the interaction between the glia and neurons, which the main components of the nervous system [2]. EVs transport proteins, lipids, and nucleic acids, thereby modifying the structural and functional aspects of the target cells during intercellular communication [3]. In the nervous system, both neurons [4] and glial cells [5] release EVs. Other studies suggest that EVs, particularly those containing microRNAs, may contribute to pain sensitization [6]. Simeoli et al. demonstrated that sensory neuron cell bodies in the dorsal root ganglia (DRG) secrete exosome-containing microRNAs (including miR-21-5p) and communicate with infiltrated macrophages after a peripheral nerve injury, leading to pro-inflammation and decreased anti-inflammatory macrophage phenotypes [6]. There is a scarcity of information regarding the release of extracellular vesicles (EVs) from satellite glial cells (SGCs) within the sensory ganglia and Schwann cells (SCs) in the peripheral nervous system (PNS), as well as their implications for nociception and pain [7]. The profiling of trigeminal SGC-shed EVs [8] showed a differentially regulated number of proteins in an in vitro model of LPS stimulation (including junction plakoglobin and myosin 9), which have been proposed as biomarkers of SGC activation under inflammatory conditions [8]. The ongoing research aims to identify and understand the specific mechanisms through which SGC-derived EVs affect pain processes. SC-derived EVs sequester TNFR1, influencing TNFα activity and regulating neuroinflammation [9] in peripheral nerve injuries. A comprehensive understanding of the roles of EVs in glia–neuron communication under both normal and pathological conditions holds the potential to uncover new

therapeutic targets and facilitate the development of innovative pain biomarkers [7,10]. Below, EVs, with a particular emphasis on peripheral glial-derived EVs, and their potential role in pain are presented, and future directions are outlined. The aim is to illustrate the state of the art of the EVs released by SCs and SGCs, with an emphasis on pain-related signaling. Moreover, the way in which such information can form the agenda for future pain research within this field and potential directions for translation towards pain management strategies are presented.

1.1. Extracellular Vesicles (EVs)

The transfer of information between cells relies on the release of diverse molecules, such as small peptides, amino acids, nucleotides, RNA, steroids, retinoids, or fatty acid metabolites. These molecules are released into the extracellular matrix either as free entities or as part of cellular vesicle cargo. EVs are structures surrounded by membranes released by most cell types [11]. Initially seen as cellular waste disposal, EVs are now recognized for having diverse functions, mediating autocrine, paracrine, and endocrine effects.

EVs are divided into exosomes, microvesicles, and apoptotic bodies based on their size, function, and biogenesis [12]. Exosomes are 30–150 nm. The International Society for Extracellular Vesicles has recommended the term small EVs (sEVs) for particles < 200 nm instead of specifying them as exosomes. This is because exosome purification will also result in the inclusion of microvesicles of comparable sizes that cannot be separated due to the lack of distinguishing markers [13,14]. Exosomes are formed inside cells, while microvesicles and apoptotic bodies are generated by external budding from the cell membrane [15].

Both glia and neurons release EVs containing proteins, nucleic acids, and lipid signaling molecules [4,5,16]. These EV cargos may influence the regulation of gene expression and downstream signaling pathways in recipient cells [16]. EV-mediated communication between glia and neurons likely leads to changes in the target cells (e.g., transcriptome or proteome in target cells), serving as a crucial contributor to nervous system disorders [17]. For example, exosomes are presented to contribute to neurodegenerative diseases [18,19], e.g., Parkinson's disease [20]; the emerging evidence highlights their involvement in regulating sensory processes, including nociception and pain [21].

Exosomes are not only involved in the normal physiological mechanisms of pain but also contribute to the development of chronic pain [7,22,23]. Unraveling the intricate details of exosome formation, cargo modification, and release in various chronic pain conditions requires dedicated research efforts. The physical stability of exosomes and the availability of advanced laboratory techniques enable their isolation and the characterization of their content to introduce avenues to discover biomarkers of therapeutic potential [24,25]. The distinctive molecules carried by sEVs in pain disorders suggest their unique potential as diagnostic biomarkers.

sEVs from mesenchymal stem cells seem promising for regenerative medicine in treating various pain conditions [26]. Additionally, changing the structure or contents of sEVs might make them a powerful method for relieving pain. Therefore, sEVs can be engineered [27] to provide therapeutic analgesic effects or to prevent chronic pain [21]. The diverse cargo of exosomes containing proteins, lipids, and RNA transcripts presents more potential advantages than traditional analgesics (e.g., opioids). This approach may mitigate adverse events, such as respiratory depression and addiction caused by systemic or local administration. However, the broader and long-term side effects of exosome-based therapies remain largely unexplored and necessitate further investigation [21]. A better understanding of sEVs in pain pathogenesis and treatment [7] will enable their clinical implementation.

Glia-divided sEVs are briefly presented below. Interested readers are encouraged to use the Gliome database (https://www.gliome.org/, accessed on 27 January 2024) as a comprehensive web-based tool to access and analyze glia secretome data [28].

1.1.1. CNS Glia-Derived EVs

EV-mediated glia–neuron intercellular communication in the central nervous system (CNS) was recently reviewed [2,10,29]. According to the available findings [7,10], glia-derived EVs and their potential role in the CNS related to the three main glial residents (astrocytes, microglia, and oligodendrocytes) are summarized below.

Astrocyte-Derived Extracellular Vesicles (ADEVs)

ADEVs play a vital role in communication between glia and neurons, contributing to neuronal maturation, survival, and the modulation of synaptic function [30]. These vesicles transport various molecules, including ATP, Hsp/Hsc70, synapsin I, FGF2, VEGF, PEDF, and endostatin. With neuroprotective capabilities against hypoxia, ischemia, oxidative stress, and hypoglycemia, ADEVs influence neuronal morphology, dendritic development, synaptic homeostasis, and neurite outgrowth. Additionally, they carry neurotoxic factors, impacting both excitatory and inhibitory synapses [10].

Microglia-Derived Extracellular Vesicles (MGEVs)

Microglia, functioning as resident macrophages, release MGEVs that are crucial for glia–neuron communication [31]. Operating in both physiological and pathological conditions, especially those related to myelin dysfunction, MGEVs modulate synaptic activity, boost excitatory transmission, and potentially contribute to mental disorders. These vesicles carry distinctive markers, regulate neuroinflammatory responses, and exhibit both detrimental and protective effects. Furthermore, MGEVs are promising potential biomarkers of chronic neurodegenerative diseases [10,32].

Oligodendrocyte-Derived Extracellular Vesicles (ODEVs)

Oligodendrocytes release ODEVs, notably exosomes, which are pivotal for bidirectional glia–neuron communication [33]. Triggered by ATP and calcium treatment, these vesicles carry myelin-related proteins. ODEVs actively contribute to maintaining axonal integrity, offering support to neurons, and facilitating fast axonal transport. They play a key role in the long-term maintenance of neurons, axonal transport, myelin diseases, and the prevention of axonal integrity loss [10,34].

Collectively, the accumulating knowledge of CNS glia-derived EVs highlights the crucial roles of these sEVs in various physiological processes and pathological conditions, proposing their potential as biomarkers and therapeutic targets for disorders within the CNS.

1.1.2. PNS-Derived EVs

A very limited amount of research is available on EV-mediated glia–neuron intercellular communication in the PNS, for instance, within the sensory ganglia of the DRG and trigeminal ganglia (TG), which calls for further research. According to the available findings, glia-derived EVs and their potential role in the PNS related to the two main glial residents (SCs and SGCs) are summarized below.

Schwann Cell-Derived Extracellular Vesicles (SDEVs)

Schwann cells (SCs) support axon maintenance and regenerative responses through various mechanisms of intercellular communication [35]. SCs play a crucial role in regulating passive axonal functions (e.g., myelin formation) and active axonal functions (e.g., sodium channel enrichment and internodal distance specifications) [36]. SDEVs are secreted by different phenotypic SCs and carry distinct proteins and nucleic acids. SDEVs mediate lateral molecular cargo transfer from SCs to axons and promote neurite sprouting and axon regeneration. They carry and transfer p53 to axons and regulate homeostasis in the peripheral nervous system (PNS) through the p75 neurotrophin receptor (p75NTR) and sortilin. SDEVs have been demonstrated to play roles in axonal regrowth, peripheral nerve repair,

and the promotion of neurite outgrowth [37]. Under pathological conditions, SDEVs are implicated in diabetic peripheral neuropathy [38] and age-related schwannomatosis [39].

The miRNAs in small EVs released by SCs have diverse effects on PNS neurons [40]. For example, mir-21a, which is released in the EVs generated by repair phenotype SCs, promotes the regeneration of targeted neurons [41]. This regeneration capacity can potentially be applicable to a PNS nerve injury and neuropathic pain. Additionally, it has been shown that SDEVs contain ample TNFR1, exhibiting reversible binding with TNFα [42]. The presence of TNFR1 in SCEVs may hold significance in initiating, sustaining, and resolving neuroinflammation by modulating TNFα activity in people with PNS injuries. Moreover, the preferential sequestration of TNFR1 could potentially alter the TNFR balance in SCs, favoring TNFR2 and consequently modifying the response to TNFα. Because TNFα directly activates nociceptors in the PNS [43], SCEVs may also emerge as exciting regulators of pain [42].

Satellite Glial Cell-Derived Extracellular Vesicles (SGC-Derived EVs)

SGCs surround the surface of neuron cell bodies in the PNS ganglia and regulate the microenvironment of the sensory ganglia. SGCs shed vesicles, including exosomes, and alter their protein profile under inflammatory conditions. The relevance of SGC-derived EVs in glia–neuron communication is an emerging area and requires further research. Our group [44] and others [45,46] are interested in unraveling the role of SGCs in pain. Collectively, these findings show that upon nerve injury or inflammation, the activated SGCs upregulate ion channels, gap junctions, and receptors, leading to neuronal excitation and pain development. The interaction of EVs and gap junctions in the nervous system seems worth investigating further, particularly in the context of pain [2,44]. Blocking gap junctions, such as through the administration of carbenoxolone, a connexin 43 blocker, has demonstrated efficacy in reducing hypersensitivity induced by chemotherapeutic agents in animal models of pain [47,48].

We have also shown that SGCs shed EVs that are the same size as exosomes (i.e., sEVs) and that the protein profile of these sEVs is altered under inflammatory conditions (in response to lipopolysaccharide, LPS) in vitro [8]. This study, which is presented below in more detail, hints at the potential role of sEV-contained proteins in inflammatory pain conditions. However, further exploration is needed to understand the precise influence of SGC-derived sEVs on neuronal excitability and pain genesis, in particular in other types of pain such as neuropathic pain or cancer pain. Exploring cellular and molecular mechanisms through the use of in vitro and in vivo models would be instrumental in unraveling the neural processes that underlie pain.

1.2. Trigeminal SGC-Derived sEVs in an In Vitro Model Mimicking Inflammatory Pain In Vivo

sEVs (i.e., exosomes) released from SGCs have been proposed to contribute to intercellular communication via miRNA and proteins. The roles of exosomes released from SGCs and their miRNA or protein cargo in nociception and pain processing are unknown. We designed and executed a series of experiments to profile both the miRNA cargo and protein cargo of these cells under normal and experimental inflammatory conditions.

1.2.1. miRNA Cargo

Our group initially outlined the characteristics of small extracellular vesicles (sEVs or exosomes) released from SGCs and their miRNA cargo in the context of nociception and pain processing. This work was presented at the European Meeting on Glial Cells in Health and Disease in Edinburgh in 2017 [49]. We profiled the miRNA expression in trigeminal SGCs and secreted exosomes following long-term activation by LPS to mimic neurogenic inflammation in vivo. We cultured fresh primary rat trigeminal ganglia SGCs obtained from adult male Sprague Dawley rats for 21 days under both normal and LPS-treated conditions. Exosomes were isolated from the conditioned media (CM) through ultracentrifugation. The global profiling of miRNAs in the SGC cell lysate identified a total

of 57 miRNAs. Upon LPS activation, several miRNAs, including a notable upregulation of miR-150, displayed significant dysregulation. MiR-150 plays a crucial role in preventing inflammation by regulating interleukin-1R-associated kinase-2. Polycystin 1 (PKD1) was identified as a predicted target for miR-150. Additionally, the CM-enriched fraction of exosomes from LPS-treated SGCs revealed more than 30 unique miRNAs. Many of these miRNAs were previously uncharacterized, contributing to a broader understanding of the regulatory responses triggered by LPS stimulation [49].

1.2.2. Protein Cargo

In 2019, our group characterized the protein cargo of small extracellular vesicles (sEVs or exosomes) released from SGCs in the context of nociception and pain processing [8]. The study successfully isolated the EVs, predominantly exosomes, from trigeminal SGCs. The size distribution of the isolated vesicles ranged from 30 to 150 nm. Myosin-9 was identified as a potential novel marker of SGC activation in the fresh primary SGCs. Other proteins, including actin, ubiquitin, fibronectin, and histone H2B, were identified in the exosomal fraction. The global screening of exosomal proteins revealed a relatively pure vesicle fraction, with the identified proteins showing protein–protein interactions. Cytoskeletal proteins likely play a role in observed morphological changes under different culturing conditions (fresh versus frozen). Five new exosomal proteins were identified, with myosin-9 identified as a potential novel marker of SGC activation. Proteins like Dsp and junction plakoglobin, associated with cell–cell adhesion, were significantly increased in the exosomes. Overall, we identified that the SGCs shed EVs in the exosomal size range, and the identified proteins have the potential to influence SGC morphology upon LPS activation. We concluded that myosin-9 could serve as a novel marker for LPS-activated SGCs in fresh primary SGCs, with potential applications in inflammatory pain conditions. A concentration of 1 ng/mL of LPSs was also found to be sufficient to activate SGCs in vitro without inducing toxicity.

2. PNS Glial-Derived sEVs and Pain: Current Status and Challenges

Aside from the studies referenced earlier [7,8,44], a recent study [50] demonstrated that the exposure of cultured DRG cells to oxaliplatin (in vitro) resulted in the production of exosomes by SGCs with a pro-nociceptive effect. After an intrathecal injection of the conditioned SGC-secreted exosomes, the naïve mice demonstrated mechanical hypersensitivity and an upregulation of TRPV1 expression in the DRG [50]. The potential mechanism underlying the observation in this study has been explained in relation to the miRNA cargo. Based on the findings [50], the authors proposed that the modification of the exosomal mechanism can act as a potential strategy for the prevention and treatment of chemotherapy-induced neuropathic pain (CINP). A limited amount of research highlights the value of studying sEVs to understand pain mechanisms and the potential targets for clinical applications, e.g., in diagnosis and therapy.

According to a review by Zhang et al. [7], most studies focus on CNS glial-derived sEVs in relation to pain. In PNS glia studies, limited evidence presents that Schwann cell-derived sEVs (SDEVs) contain miR-23b-3p, which, after mechanical stimulation, targets neuropilin 1 in neurons, aiding peripheral nerve injury repair. The proteomic analysis of SDEVs has also revealed proteins associated with axon regeneration and anti-inflammatory effects in neuroinflammation. Therefore, a potential role exists for SDEVs in pain. However, challenges include improving the culture conditions for optimal sEV efficacy and addressing heterogeneity and limitations in manufacturing substantial sEV quantities for repair and therapeutic purposes.

The current general challenges related to sEVs are mainly related to the identification and characterization of sEVs. sEVs are formed through distinct mechanisms involving intracellular membrane trafficking pathways and exhibit a rich and diverse composition. The precise identification of sEV subtypes is challenging due to the absence of specific markers. Currently, there is no universally accepted gold standard method for isolating sEVs. Vari-

ous techniques are employed, such as ultracentrifugation, density gradient centrifugation, ultrafiltration, size-exclusion chromatography, and combinations of these methods. Novel approaches, like magnetic-bead-mediated selective adsorption and microfluidics, have also emerged. However, differential ultracentrifugation, density gradient centrifugation, and size-exclusion chromatography remain the most commonly used methods, each with their limitations in simultaneously achieving a high yield and strong purity. Ultracentrifugation involves using different gradients during the initial steps and ultra-high-speed centrifugation, resulting in moderate purity. Density gradient centrifugation utilizing separation media like sucrose and iodixanol yields improved purity but with less production compared to ultracentrifugation. Size-exclusion chromatography separates vesicles based on size, while ultrafiltration obtains larger vesicles with low-level purity. Immunomagnetic separation captures sEVs using antibodies and magnetic beads, ensuring high purity but at a higher cost. Microfluidic technology controls the fluid flow to separate and capture sEVs, requiring specialized equipment. Commercial kits offer a simple procedure, but the quality of isolated sEVs varies. Due to the sEVs' inherent heterogeneity, the isolation method significantly influences their components and function. There is an urgent need for a systematic approach to evaluating yield and purity, aiming to establish a universally accepted gold-standard extraction method. In addition, characterization techniques include electron microscopy for morphology, nanoparticle tracking analysis for size distribution, and Western blotting, immunofluorescence, or flow cytometry for protein markers, which also require standardization and harmonization.

sEVs serve as ubiquitous paracrine communication tools, transporting biomolecules to modulate gene expression and function over short and long distances. An advantage of sEVs is their ability to cross the blood–brain barrier (BBB) and blood–spinal cord barrier (BSB), thereby enabling access to remote targets throughout the nervous system. Recent progress has illuminated the interplay among neurons and glial, immune, and tissue cells, all of which are engaged in managing pain signaling, where sEVs derived from both the central and peripheral tissues have been associated with these mechanisms [7]. Therefore, this potential needs to be fully explored. Below are several proposed directions for the future use of sEVs, with a focus on pain research and management.

3. Future Directions for sEVs in Pain

3.1. Biomarkers

There is potential for sEVs to be used as biomarkers of clinical pain disorders. Currently, this field is facing huge challenges in objectively assessing persistent pain, and using sEVs as biomarkers could be advantageous. This is proposed based on the ability of sEVs to traverse barriers, encapsulate essential components, and carry molecules that are relevant to pain conditions. For example, miRNAs in sEVs associated with disc herniation have been reported in the literature. The increased expression of sEVmiR-223 during the acute phase of disc herniation has been associated with a decreased risk of radicular pain [51]. SEVs also exhibit potential as prognostic indicators for pain management; for example, lower levels of sEV-miR-338-5p have been associated with a poor response to the treatment of chronic neuropathic pain [52]. The findings suggest that sEVs hold promise as tools in pain treatment, but technological challenges and the need for further investigations remain. Advanced single-vesicle technologies are recognized as potential solutions for the precise and noninvasive detection of sEV biomarkers.

3.2. Pain Relief through MSC-sEVs

Regenerative medicine has emerged as a promising approach for treating neurological disorders, including spinal cord injuries, strokes, Alzheimer's disease, and traumatic brain injuries [53]. Notably, stem cell therapy has gained significant attention in the field of pain [54]. Mesenchymal stem cells (MSCs) derived from various sources, such as induced pluripotent stem cells (iMSCs), bone marrow (BMSC), umbilical cords (UCMSC), adipose tissue (ADMSC), and dental pulp (DMSC), have demonstrated potential therapeutic effi-

cacy [55]. MSC-sEVs present a promising alternative to conventional cell-based therapies, offering advantages such as an immunogenicity absence, a lack of neoplastic qualities, and the ease of preservation. Additionally, they can cross the blood–brain barrier (BBB). With the ability to protect contents from degradation, such as miRNA, and their various delivery modes, including intravenous, intrathecal, intranasal, and local delivery, MSC-sEVs demonstrate versatility. These attributes position MSC-sEVs as a highly promising therapeutic option, particularly for pain relief, potentially substituting traditional cell-based therapies.

The diverse therapeutic applications of sEVs derived from MSCs in pain management have recently been reported in the literature [7,55–57]. However, it is crucial to highlight that the safety and efficacy of MSC-sEVs remain to be elucidated. Here are a few examples:

1. Local delivery in tendinopathy and osteoarthritis: The continuous local delivery of MSC-sEVs in rats mitigates tendinopathy-associated acute pain. MSC-sEVs reduce mast cell infiltration, lower proinflammatory cytokines, and alleviate osteoarthritis pain [58].
2. Neuropathic pain interventions: UCMSC-sEVs demonstrate dose-dependent analgesic effects for those with neuropathic pain. The intrathecal administration of MSC-sEVs prevents neuropathic pain development and improves patients' pain thresholds. Wrapped UCMSC-sEVs in an alginate scaffold provide prolonged antinociceptive effects and promote axon regeneration. MSC-sEVs influence neuron and glial activation, downregulate Rsad2 expression, and inhibit TLR2/MyD88/NF-κB signaling. Encapsulated miRNAs in MSC-sEVs, such as miR-26a-5p, miR-99b-3p, miR-190b-5p, and miR-152-3p, regulate microglia activation and suppress neuroinflammation [7,59].
3. Diabetic peripheral neuropathy (DPN): The systemic administration of MSC-sEVs in a murine DPN model restored mechanical and thermal thresholds and caused M1-to-M2 macrophage phenotype shifting. MSC-sEVs enhance nerve conduction velocity and support the survival and axonal growth of injured neurons in DPN [7,60].
4. Myelination and central diseases: MSC-sEVs promote myelination in injured neurons and contribute to the restoration of neural function in central diseases like spinal cord injuries [61], stroke, and multiple sclerosis [7].
5. Visceral pain and immunomodulation: MSC-sEVs alleviated chronic pelvic pain in an experimental autoimmune prostatitis (EAP) model [62] by modulating immune cell counts and reducing COX-2 overexpression. MSC-sEVs demonstrate therapeutic potential [7] for pain associated with inflammatory bowel disease (IBD) and cystitis by regulating immune responses and promoting the M2 macrophage phenotype.

3.3. Modification of sEVs for Targeted Pain Management

sEVs are being explored as vehicles for optimized molecule delivery in pain management. Various modification approaches have been examined, such as engineered sEVs, biomaterial combinations, and artificial EV creation, to facilitate drug loading and enhance specificity and targetability. Modified sEVs aim to effectively deliver bioactive molecules, minimizing the off-target effects in pain pathways. sEVs offer advantages like low-level immunogenicity, high biocompatibility, and enhanced stability over traditional drug delivery systems. The engineered approaches involve modifying producer cells or isolated sEVs to augment generation and impart distinct biomolecules. Methods include endogenous (parent cell modification) and exogenous (direct sEV modification) approaches. The challenges of the engineering of EVs are related to their direct use, such as insufficient targeting, efficacy, and dosages. Engineering strategies to optimize EVs for therapeutic purposes include surface modification, loading specific therapeutic agents, and genetic engineering. The application of engineered EVs in regenerative medicine has recently been summarized, and two categories of engineered EVs have been marked: those produced by donor cells and directly engineered EVs [7,63].

EVs' characteristics make them feasible candidates for delivering specific molecules, such as therapeutic drugs, to targeted tissues. These applications are relevant for many disorders. One particular area is neurophysiological research and the investigation of

neurological disorders, including CNS tumors, autoimmune conditions, and neurodegenerative diseases [64]. One example of such an approach has been described for Amyotrophic Lateral Sclerosis (ALS) [65]. In ALS pathogenesis, EVs are produced by various cell types in the CNS and neuromuscular junctions. Specifically, in ALS, these EVs may transport disease-related molecules (proteins and miRNAs), contributing to the transformation and degeneration of the brain and neuromuscular elements. This process facilitates the spread of pathology among different cell types and allows the EVs to travel over long distances in the body, facilitating the exchange of harmful molecules between the brain and neuromuscular junctions. Considering this, the molecules carried by EVs circulating in the bloodstream and cerebrospinal fluid are regarded as potential biomarkers for diagnosing and predicting the prognosis of ALS. Moreover, EVs exhibit therapeutic potential. By impeding the transfer of EVs carrying harmful molecules and administering EVs loaded with neuroprotective cargo, the progression of ALS may be decelerated, or its pathological effects may even be reversed. In essence, this dual role of EVs in ALS suggests their significance as both contributors to the disease process and potential avenues for therapeutic interventions [65]. This model offers valid points related to pain and pain relief.

Recent studies have concentrated on the joint application of sEVs with biological biomaterials. For example, researchers designed photocrosslinkable alginate hydrogels that incorporate fibronectin to encapsulate, anchor, and retain engineered sEVs for seven days, preserving both their structural integrity and functionality [66]. A novel approach involves employing a porous alginate scaffold to entrap combinatorial MSC-sEVs for pain management. This technique demonstrated significant analgesic effects in neuropathic pain models, maintaining efficacy for up to 21 days. In contrast, single doses of MSC-sEVs were rapidly cleared and provided only short-term pain relief, as indicated by previous studies [67]. The alginate scaffold delays the release of MSC-sEVs, thereby prolonging their effectiveness and balancing anti-inflammatory and pro-inflammatory mediators in the DRG [67]. Similarly, gels composed of PDLLA-PEG-PDLLA triblock copolymers (PLELs) loaded with circRNA3503-overexpressing MSC-sEVs (PLEL@circRNA3503-OE-sEVs) have shown promise in treating osteoarthritis, particularly in cartilage tissues [68]. The PLELs gradually release the circRNA3503-loaded sEVs, exerting a therapeutic effect [68]. These findings suggest that the combination of sEVs with biological biomaterials may offer a strategy to enhance the therapeutic effectiveness of sEVs in pain disorders.

Collectively, modified sEVs are promising in personalized medicine, with potential applications in various pain disorders. The challenges include overcoming the efficiency issues and the need for methods that do not compromise the sEVs' integrity.

Recently, nanovesicles (NVs) have been mechanically produced with a higher yield and can serve as alternatives to sEVs [69]. NVs share traits like drug encapsulation with sEVs but have different protein profiles. NVs loaded with analgesic drugs could theoretically present a novel therapeutic approach for pain management, among other nanotechnology-based management techniques [70]. However, the potential of NVs as therapeutic agents remains largely unexplored, lacking a comparative analysis with EVs.

4. Conclusions

Glial cells play a critical role in maintaining proper neuronal function under normal conditions and step in to restore balance when the nervous system faces challenges from environmental or pathological factors. The communication between glial cells and neurons is vital, and extracellular vesicles (EVs) contribute to this process by carrying a diverse array of molecules capable of influencing the function of the recipient cells. sEVs (e.g., exosomes) are released from both neurons and glial cells in both the CNS and the PNS and play a critical role in their physiology and pathophysiology.

A limited amount of evidence demonstrates the potential roles of SGC-derived small sEVs in pain. Our group and others attempted to clarify the role of SGCs in chronic pain, exploring their morphology, molecular markers, and physiological functions. In the same vein, a limited amount of information has been provided related to sEVs derived from SGCs

of the PNS. We showed that sEVs from trigeminal SGCs present miRNA [49] and protein [8] cargos related to an inflammatory model of pain in vitro. Zhao and colleagues [50] showed the role of sEVs (miRNA cargo) from dorsal root SGCs in the modulation of CINP.

Cytokines are known as regulators of responses to PNS injuries. TNFα is expressed early during the course of PNS injury and is important in orchestrating the subsequent expression of many secondary cytokines [43]. SCEV research suggests that EVs may provide a novel layer of regulatory control for PNS injuries. SCEV-associated TNFR1 has been proposed to function as a powerful TNFα decoy in the injured PNS. The activity of SCEVs in TNFα regulation may be bimodal. Because the association of TNFα with EV-associated TNFR1 is reversible, when the concentration of soluble TNFα begins to decrease in the injured PNS microenvironment, TNFα may be released from the SCEVs, causing the decrease in free TNFα to be less precipitous. TNFα directly activates nociceptors in the PNS, and hence, SCEVs may emerge as exciting regulators of pain states. The unanswered questions in this field will shape future research. For example, it remains to be determined if SCEVs can regulate PNS injuries.

Further investigations into how glial cells function as a system, their interactions with neural networks, and their influence on physiological and pathological processes could provide valuable insights into the mechanisms underlying neurological disorders. The ultimate goal is to use this knowledge to identify potential therapeutic targets or biomarkers for the diagnosis or treatment of neurological disorders.

While sEVs show potential as biomarkers and therapeutic targets for pain, their precise mechanisms and diagnostic validity require validation. Modified sEVs for pain relief are emerging, but more research into cargo and targeted modifications is needed to unlock their full therapeutic potential. Although the role of sEVs in pain pathogenesis, diagnosis, and treatment is being established, significant knowledge gaps persist. In-depth research on sEVs' effects on pain is crucial for unlocking their full potential and developing innovative and less-invasive pain management therapies. In this context, any exploration of sEVs, irrespective of their source (such as neurons, glial cells, or other tissue types in the PNS and CNS), would be extremely advantageous. This approach contributes to a more comprehensive understanding of the intricate aspects involved in targeting pain.

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