International Journal of Nanomedicine

Dovepress
Taylor & Francis Group

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REVIEW

Source-Specific Extracellular Vesicle Functions and Engineering Strategies for Chronic Pain Management: A Comprehensive Review

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Abstract: Chronic pain management faces significant limitations due to adverse effects and insufficient long-term relief from existing therapies. Extracellular vesicles (EVs) are lipid bilayer-enclosed particles naturally carrying proteins, nucleic acids, and metabolites. Recently, EVs have emerged as a potential alternative approach. This review examines EVs from mesenchymal stem cells, neural cells, macrophages, and gut microbiota. EV activity is then assessed across the three major pain types defined by the ICD-11: nociceptive pain, neuropathic pain, and nociplastic pain. We elucidate how source-specific EVs dynamically regulate different kinds of pain through multi-modal mechanisms, including neural signal transduction, neuroimmune axis coordination, structural neural repair, and metabolic network reprogramming. Furthermore, we discuss how these inherent therapeutic properties can be augmented through engineering approaches such as surface modification and cargo encapsulation, which enhance targeting and payload delivery. By integrating mechanistic insights into source-specific EV functions with emerging engineering strategies, this review may provide a rational framework for developing next-generation EV-based analgesics. We conclude that harnessing the innate biological properties of EVs, complemented by strategic engineering, represents a potential non-opioid strategy for precise and effective management of chronic pain.

Keywords: exosomes, non-opioid treatment, EV-based analgesic therapy, engineering EVs, neuroimmune modulation, neuropathic pain

Introduction

Pain is a prevalent global health issue, imposing a substantial socioeconomic burden and severely compromising quality of life. Global estimates suggest that approximately 30% of the population experiences chronic pain.[1] Long-term survey data points to a stark economic divide between those with and without chronic pain. The cumulative cost difference approaches €55,000 per person. When scaled nationally, the annual burden reaches approximately €12 billion, equivalent to around 4% of gross domestic product (GDP). Productivity losses make up nearly 80% of this total.[2] According to the International classification of diseases (ICD-11),[3] pain can be classified into three main types: nociceptive, neuropathic, and nociplastic.[4] Current analgesic strategies, including nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and neuromodulation techniques, are limited by systemic toxicity, addiction risks, and limited efficacy against chronic pain.[5] This therapeutic dilemma has driven the search for novel biological vectors beyond conventional pharmacological interventions, with extracellular vesicles (EVs) emerging as promising candidates for analgesia.

https://doi.org/10.2147/IJN.S598699
Received: 24 January 2026
Accepted: 2 May 2026
Published: 8 May 2026

EVs, lipid bilayer-enclosed particles, are a heterogeneous group of cell-derived membranous structures and recognized as key mediators of intercellular communications. Virtually all cell types within the human organism actively release EVs.[6] Their molecular cargo, selectively packaged proteins, nucleic acids, and lipids is delivered to recipient cells through autocrine, paracrine, or endocrine signaling. This sophisticated transport system enables EVs to influence pain pathophysiology through several key mechanisms: neural signal modulation, neuroimmune axis regulation, neuroprotection, and metabolic regulation in pain circuits.

In recent decades, accumulating evidence has highlighted the critical role of EVs in various chronic pain conditions, particularly osteoarthritis (OA), rheumatoid arthritis (RA), and chemotherapy-induced peripheral neuropathy (CIPN).[7] Different forms of EVs have shown remarkable therapeutic potential for pain management. Unlike traditional analgesics, EVs offer inherent biocompatibility, cell-type specificity, and adaptability to microenvironmental cues, allowing them to engage in both pathological and physiological processes simultaneously. Nevertheless, significant translational hurdles temper the therapeutic promise suggested by these associations. EV-based interventions currently face several practical bottlenecks. Scalable and reproducible manufacturing remains difficult to achieve. Batch-to-batch variability, incomplete knowledge of in vivo fate and off-target deposition, and an immature regulatory landscape further compound the challenge. The gap between encouraging preclinical findings and standardized clinical application is therefore considerable. Acknowledging these constraints is necessary to avoid overstating the field's present state of readiness.

Engineering strategies, such as surface modification and cargo encapsulation, can enhance these advantages by optimizing biodistribution, cargo loading, and cellular uptake precision. They offer a potential means to overcome some limitations of native vesicles, yet they introduce their own set of obstacles. Surface modification and cargo encapsulation can enhance targeting and potency. Yet translation to industrial production under good manufacturing practice (GMP) standards remains challenging. Manufacturing consistency, purification efficiency, and cost are persistent concerns. Systemic delivery also requires overcoming biological barriers and rapid clearance. Progress will depend on standardized production platforms and responsive vesicle designs. These translational gaps underscore the need for a critical evaluation of current engineering approaches.

Several reviews have examined facets of EV-mediated analgesia in recent years. Some have broadly surveyed the therapeutic potential of EVs in pain, while others have concentrated on specific subfields, most notably the mechanisms of MSC-derived EVs in neuropathic pain models. Although these contributions have advanced the field, they largely treat cell sources, pain categories, and engineering strategies in isolation. A systematic integration that compares EV functions across distinct cellular origins and maps them onto the three ICD-11 pain classifications has not been undertaken. Moreover, the interplay between native EV heterogeneity and the design of engineered variants remains underexplored in the existing literature. The present review addresses these specific gaps. By adopting an explicit source-comparative framework and maintaining a consistent focus on the three pain categories, it aims to provide an organizational contribution that complements rather than duplicates prior work.

Therefore, to address these unmet needs, this review provides a comprehensive synthesis of EVs heterogeneity across pain etiologies and their roles in neuro-immune-metabolic crosstalk and evaluates cutting-edge bioengineering strategies to overcome delivery and scalability challenges. By integrating mechanistic insights across pain subtypes and therapeutic development challenges, this work aligns with the NIH's call for mechanism-based non-opioid analgesics. The aim is to help position EV therapeutics as a clinically viable direction for precise pain management and to inform future translational efforts.

This manuscript is well-organized as follows. Biological Characteristics of EVs: Source-Specific Functional Profiling covers the biological characteristics of EVs, with an emphasis on source-specific functional profiling. Section 3 then turns to the role of EVs in pain pathogenesis. A mechanistic synthesis across the three ICD-11 pain classifications is provided there. Molecular Mechanisms of EV-Based Analgesia Therapy examines the molecular mechanisms that underpin EV-based analgesic therapy. In Potential Applications of Engineering EVs in Pain Treatment, the potential applications of engineering EVs in pain treatment are evaluated. Conclusions and Further Perspectives offers conclusions and outlines further perspectives for the field.

Biological Characteristics of EVs: Source-Specific Functional Profiling

EVs are systematically classified into exosomes (50–150 nm), microvesicles (100–1000 nm) and apoptotic bodies (1000–5000 nm) based on three biogenetic pathways. Apoptotic bodies arise from programmed cell death. Exosomes form when cellular membranes invaginate, creating intraluminal vesicles (ILVs) inside multivesicular bodies (MVBs). These MVBs fuse with the plasma membrane or Golgi apparatus, releasing ILVs as exosomes through endosomal sorting complex required for transport (ESCRT)-dependent or -independent pathways. Microvesicles are released through calcium-dependent plasma membrane budding, enclosing nearby biomolecules.[8] EVs precisely regulate recipient cell functions through three distinct delivery mechanisms.[9] First, receptor-mediated signaling activation occurs when membrane-embedded proteins on EVs interact with specific receptors on target cells, initiating downstream signaling cascades. Second, membrane fusion-driven cargo delivery allows EVs to release their luminal contents directly into the cytoplasm through lipid bilayer fusion with the host cell membrane. Lastly, precise endocytic trafficking enables cellular uptake via endocytosis, directing EV cargo to specific subcellular compartments for localized functional modulation. The functional diversity of EVs in disease regulation is inherently determined by their cellular origin. The parental cell type imparts unique molecular signatures on EVs, enabling precise modulation of distinct cell-to-cell pathways. Given the complex role of EVs in disease progression, many researchers have explored the use of EVs from diverse sources for therapeutic applications. This section systematically examines the biological characteristics of EVs derived from various sources, supported by experimental evidence and mechanistic insights (Table 1 and Figure 1).

Stem Cell-Derived EVs

Stem cells offer significant potential in regenerative therapeutics due to their multipotent differentiation capabilities. However, clinical applications of whole stem cell transplantation face critical limitations, including immunogenic rejection risks and potential tumorigenesis linked to genomic instability during prolonged in vitro expansion.[47] These challenges, particularly the emergence of chromosomal abnormalities and epigenetic alterations in cultured stem cells, have redirected scientific attention toward their secreted EVs. Compared to parental stem cells, stem cell-derived EVs retain therapeutic bioactivity while circumventing ethical concerns, minimizing immunogenic responses, and eliminating risks of uncontrolled proliferation. Their innate biocompatibility, potential for scalable production, and ability to bypass

Table 1 Application of Different Types of Extracellular Vesicles in Pain

Cell Source	Model/Pain Type	Cargo	Proposed Molecular Mechanism	Function/Therapeutic Effects	Reference
Adipose-derived stem cell extracellular vesicles (ADSC-EVs)	OA	miR-21-5p	Reduce synovial IL-6 and TNF-α levels, attributed to EV-transferred TGF-β1-mediated suppression of NF-κB signaling in macrophages	Enhance anti-inflammatory mediator expression and reprogramming	[10]
	OA	HSP70	Inhibit NLRP3 inflammasome assembly and caspase-1-dependent IL-1β maturation	Attenuate spinal microglial activation	[11]
	SCI	miR-21-5p	Reduce oxidative stress in dorsal horn neurons via miR-21-5p-mediated PTEN/Akt pathway activation	Exhibit significant neuroprotective and axonal regenerative capacities	[12]
	RA	FasL	Induce apoptosis of activated CD68+ synovial macrophages, reducing synovial infiltration.	Immune regulation and bone protection	[13]
	Type 2 diabetic	NADH dehydrogenase subunits	Increase ATP synthesis and mitigate metabolic stress.	Mediate metabolic regulation	[14]

(*Continued*)

https://doi.org/10.2147/IJN.S598699

Table 1 (Continued).

Cell Source	Model/Pain Type	Cargo	Proposed Molecular Mechanism	Function/Therapeutic Effects	Reference
Umbilical cord mesenchymal stem cell-derived extracellular vesicles (UC-MSC-EVs)	CCI	lncRNA UCA1	Bind and sequester miR-96-5p to relieve its repression of FOXO3a; reduce spinal microglial activation, lowering pro-inflammatory mediators.	Suppress neuroinflammatory cascades (reverses mechanical allodynia and thermal hyperalgesia)	[7]
	Spinal nerve ligation	GABA	Normalize GABA balance in dorsal horn neurons.	Prevent central sensitization	[7]
	OA	miR-1208	By polarizing macrophages to an M2 phenotype, reduce synovial PGE2 levels and improve cartilage integrity.	Attenuate synovial inflammation	[15]
	CCI	miR-181c-5p	Suppresses MYD88-dependent NF-κB/NLRP3 signaling, reducing IL-1β and COX-2 production and mitigating blood-spinal cord barrier dysfunction	Regulate immune cells and enhance the anti-inflammatory microenvironment	[7]
Bone marrow mesenchymal stem cell-derived extracellular vesicles (BMSC-EVs)	SCI	miR-21-5p and miR-222-3p	Promote DRG neurite outgrowth and restore corticospinal tract integrity by activating the PI3K/Akt/mTOR pathway	Ameliorate axon regeneration to alleviate pain	[16]
	OA	miR-127-3p	Reduce IL-6 and TNF-α production and inhibit NLRP3 inflammasome activation	Anti-inflammatory effect	[17]
	RA	FasL/Fas	Increase Treg apoptosis EVs expand Tregs via TGF-β1/IL-10 signaling	Exhibit immunosuppressive properties	[18]
	RA	TGF-β1	Downregulate NKG2D expression, impairing granzyme B release and synovial infiltration	Inhibit NK cell effector functions	[19]
Induced pluripotent stem cell-derived mesenchymal stem cell extracellular vesicles (IMSC-EVs)	Tendinopathy	Annexin A1 and miR-124-3p	Polarize synovial macrophages from pro-inflammatory M1 to anti-inflammatory M2 phenotypes	Reprogram macrophage phenotypes to establish an anti-inflammatory microenvironment	[20]
	SCI	DUSP2 and DUSP3	Dephosphorylate p38 MAPK, reducing IL-1β and TNF-α release	Resolve neuroinflammation by silencing central sensitization pathways	[21]
	OA	RvD1 and GDNF	Downregulate TRPV1 and ASIC3 expression in sensory nerve endings	Desensitize peripheral nociceptors through targeted ion channel modulation	[22]
Neuron-derived extracellular vesicles (NDEVs)	OA	miR-146a-5p	Suppress NF-κB activation in synovial macrophages; reduce pro-inflammatory factors IL-1β and PGE2 while expanding Tregs	Serve as bidirectional mediators of neuro-immune interactions to fine-tune inflammatory pain	[10]
	DPN	Oxidized mitochondrial DNA and NADH dehydrogenase subunits and HSP70	Restore electron transport chain integrity; repaired mitochondrial function rescues energy metabolism while alleviating oxidative stress-induced pain	Modulate mitochondrial dysfunction and metabolic stress	[23–25]

(*Continued*)

Table 1 (Continued).

Cell Source	Model/Pain Type	Cargo	Proposed Molecular Mechanism	Function/Therapeutic Effects	Reference
Microglia-derived extracellular vesicles (MDEVs)	Chemotherapy-induced neuropathy	miR-219	Transfer pro-remyelination factors to oligodendrocyte precursor cells. This transfer drives OPC differentiation into mature myelinating oligodendrocytes	Restore saltatory conduction while reduce ectopic firing in pain pathways	[26]
	TBI	miR-155	Suppress SOCS1 expression in spinal glia. This suppression amplifies JAK/STAT3 signaling and increases glial-derived neurotrophic factor release	Escalate synaptic strength and stabilize pain-related neural circuits	[27]
	SCI	Netrin-1 and Semaphorin 3A	Activate PI3K/Akt/mTOR pathways to stimulate DRG neurite outgrowth, facilitating functional recovery	Promote axonal regeneration	[28]
Astrocyte-derived extracellular vesicles (ADEVs)	SCI	miR-21	Suppress JAK2/STAT3 signaling in astrocytes and reduce glial scar formation	Enhance remyelination and promote neuronal survival	[29]
	Bone cancer pain	miR-146a	Inhibits NF-κB signaling in bone cancer pain, reducing pro-inflammatory cytokine release	Drive central sensitization	[30]
Oligodendrocyte-derived extracellular vesicles (ODEVs)	Central post-stroke pain	Neurofilament proteins	They stabilize microtubule networks via RhoA/ROCK pathway modulation	Provide axonal support	[31]
	MS	PLP	Restore saltatory conduction while reducing ectopic firing in nociceptive pathways; alleviate neuropathic pain	Facilitate remyelination in demyelination-associated pain conditions	[32]
Schwann cell-derived extracellular vesicles (SC-EVs)	CCI	miR-142-5p	Suppress ACTN4 and ELAVL4, destabilizing actin filaments and mRNA stability in dendritic spines	Regulate neuroinflammatory processes in chronic pain states	[33]
	SCI	Neurofilament proteins	Enhance cytoskeletal stability while activating PI3K/Akt/mTOR/p70S6 kinase pathway	Drive axonal regrowth	[34]
	DPN	miR-142-5p	Target ACTN4 and ELAVL4 in hippocampal neurons	Impair synaptic plasticity and exacerbate cognitive-pain comorbidities	[35]
Bacterial Extracellular Vesicles (BEVs)	CIPN	Lactate	Maintain ATP production, ultimately preventing axonal degeneration	Promote axonal integrity via cargo-mediated metabolic and cytoskeletal regulation	[36]
	Osteonecrosis	Lactobacillus animalis-derived Evs	Traverse intestinal barriers, enter systemic circulation, and accumulate in femoral bone tissue	Coordinate multi-organ communication through the gut-bone-brain axis	[37]
	RA	PG	Activate synovial fibroblasts through TLR/NOD receptors, upregulating MMPs and cytokines	Enhance systemic innate immunity	[38]
	OA	LPS	Increase intestinal permeability, enter circulation, and activate joint tissue macrophages via TLR4 priming	Mitigate neuroinflammation in pain-processing regions	[38]

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https://doi.org/10.2147/IJN.S598699

Table 1 (Continued).

Cell Source	Model/Pain Type	Cargo	Proposed Molecular Mechanism	Function/Therapeutic Effects	Reference
Macrophage-Derived EVs (MDEVs)	DPN	GAD65	Convert excitatory glutamate to inhibitory GABA; restore spinal GABAergic tone and rebalances excitatory-inhibitory equilibrium	Provide metabolic and structural support to compromised sensory neurons	[39]
	CNS trauma	miR-219	miR-219-mediated ROCK inhibition stabilizes microtubule networks to reverse axonal transport deficits	Promote axonal repair	[40]
Tumor-derived extracellular vesicles (TDEVs)	Cancer pain	miR-155	Suppress SOCS1, amplifying TLR4/NF-κB signaling and NLRP3 inflammasome assembly; drive IL-1β and TNF-α release, potentiating central sensitization	Activate glial-immune crosstalk to sustain neuroinflammation	[41]
	Cancer pain	MMPs 1 and thrombin-sensitive protein 1	Activate receptor 1 by activating a protease; activate the protease-activating receptor 2 and the F2R-like trypsin receptor 1	Augment the sensitivity of nociceptors to nociceptive stimuli	[42]
Plant-derived extracellular vesicles (PDEVs)	IBD	Ginseng-derived nanoparticles	Alleviate IBD via p62/Nrf2/Keap1 pathways and inhibit TLR4/MAPK-mediated pro-inflammatory signaling like ZO-1, occludin	Modulate inflammatory pathways through dual suppression of innate immune signaling and activation of cytoprotective cascades	[43]
	RA	Folic acid	Remodel immune microenvironment in RA via the PI3K-AKT pathway	Reprogram immune microenvironment through targeted cellular modulation and pathway intervention	[44]
Serum-derived extracellular vesicles (SEVs)	SCI	Leucine enkephalin	Increase the CD206 + macrophages	Induce short-term mechanical anti-pain	[45]
	CIPN	Serum	Inhibit glial responses and improve nerve conduction after CIPN	Relieve inflammatory reaction to alleviate pain	[46]

Abbreviations: CIPN, chemotherapy-induced peripheral neuropathy; RA, rheumatoid arthritis; OA, osteoarthritis; SCI, spinal cord injury; IBD, inflammatory bowel diseases; DPN, diabetic peripheral neuropathy; GABA, γ-aminobutyric acid; NLRP3, NOD-like receptor thermal protein domain associated protein 3; TLR4, toll-like receptor 4; ATP, adenosine triphosphate; LPS, lipopolysaccharide; PLP, proteolipid protein; MS, multiple sclerosis; CCI, chronic constriction injury; TRPV1, transient receptor potential vanilloid 1; NF-κB, factor-kappa B; TBI, traumatic brain injury; DRG, dorsal root ganglion; HSP70, heat shock protein 70; PGE2, prostaglandin E2; TGF-β1, transforming growth factor-β1; MMPs, matrix metalloproteinase; MAPK, mitogen-activated protein kinase; MYD88, myeloid differentiation factor 88; TNF-α, tumor necrosis factor-alpha; NADH, nicotinamide adenine dinucleotide.

cellular engraftment barriers position EVs as a transformative approach in regenerative medicine. They offer targeted therapeutic effects without compromising systemic safety.[48]

Adipose Stem Cell-Derived EVs

Adipose-derived stem cell extracellular vesicles (ADSC-EVs) are nanoscale lipid bilayer particles secreted by adipose-derived mesenchymal stem cells (ADSCs), which reside abundantly in subcutaneous and visceral adipose tissue. These EVs inherit molecular profiles from their parental cells, including surface markers such as cluster-of-differentiation antigen 9 (CD9), adhesion molecules, and immunomodulatory proteins like transforming growth factor-β (TGF-β) and tumor necrosis factor-stimulated gene 6 (TSG-6). Their cargo is enriched with anti-inflammatory microRNA (miRNA), growth factors such as insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF), and metabolic regulators like adiponectin and leptin. The cargo enable them to mediate intercellular communication across diverse tissues. ADSC-EVs are generated through two primary biogenetic pathways, ESCRT-dependent exosome sorting

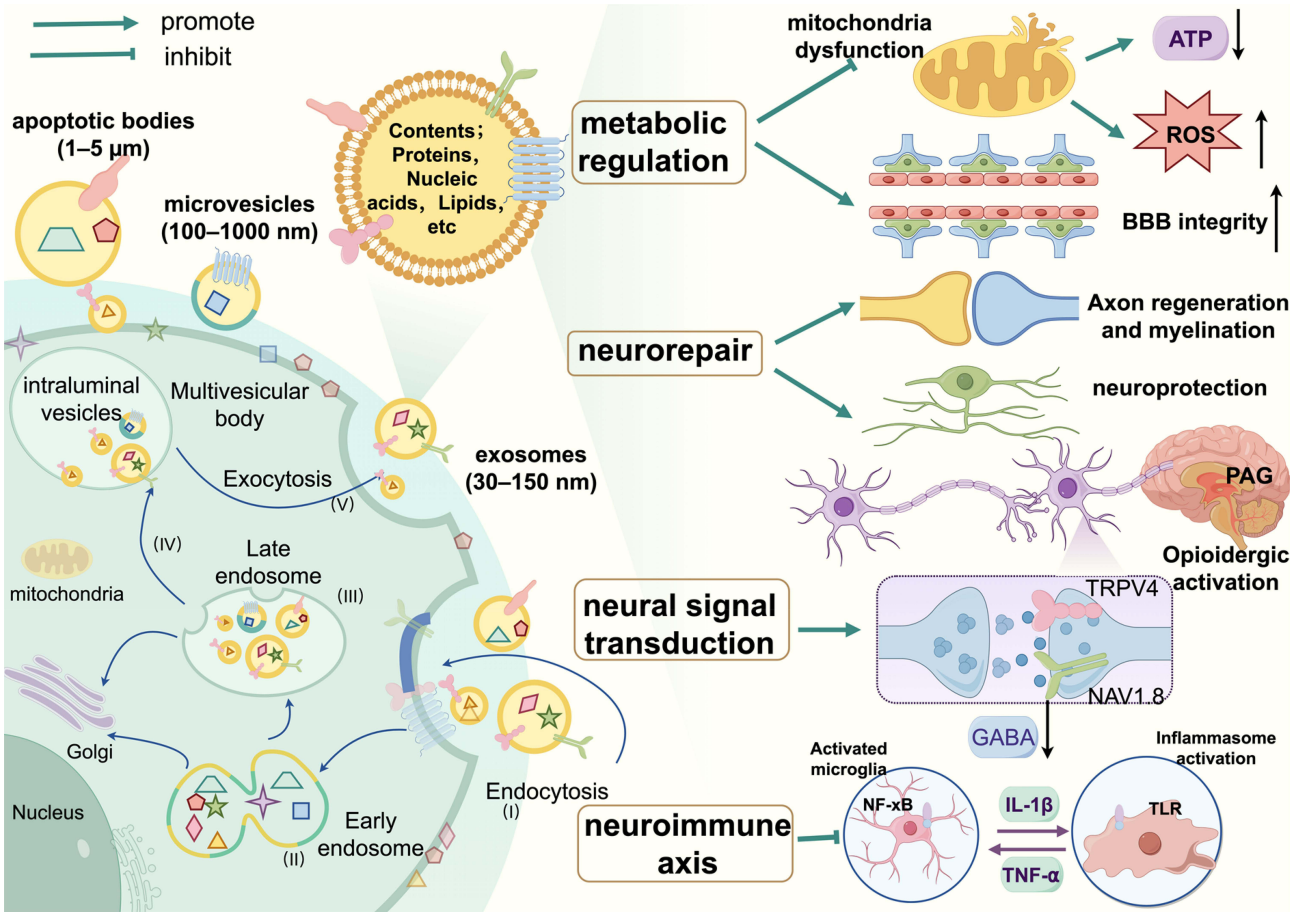

Figure 1 Biogenesis, composition of EVs and its roles in the pathogenesis of pain.
Abbreviations: ATP, adenosine triphosphate; TRPV4, transient receptor potential vanilloid 4; PAG, periaqueductal gray; TNF-α, tumor necrosis factor-alpha; IL-16, interleukin-16; Nav1.8, voltage-gated sodium channels; GABA, γ-aminobutyric acid; TLR, toll-like receptor; NF-κB, factor-kappa B; ROS, reactive oxygen species; BBB, blood-brain barrier.

and calcium-triggered plasma membrane budding. Their release is dynamically regulated by microenvironmental cues such as hypoxia, inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), and mechanical stress, ensuring context-specific functional adaptation.[11]

Preclinical studies have indicated the therapeutic potential of ADSC-EVs in various pathologies, intimately linked to chronic pain, including metabolic disorders, autoimmune diseases and neurodegenerative diseases.[49] This relevance stems from their core biological capabilities. First, ADSC-EVs resolve insulin resistance and peripheral neuropathy in type 2 diabetes via adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) activation.[50] Besides, they suppress inflammatory cascades in Crohn's disease (CD) through macrophage reprogramming and NOD-like receptor thermal protein domain associated protein 3 (NLRP3).[51] Neurodegenerative diseases also benefit from ADSC-EVs. In Parkinson's disease (PD), EV-delivered miR-21-5p inhibits microglial activation and neuroinflammation, promoting neuronal survival and motor function preservation.[52] Their conserved functions in promoting angiogenesis, countering oxidative stress,[53] and modulating immune responses[54] collectively establish a multifunctional platform capable of simultaneously targeting inflammatory, neuropathic, and metabolic components of pain. This functional versatility provides the mechanistic foundation for their targeted application in specific pain states, as detailed in the following analysis of preclinical pain models.

ADSC-EVs exert targeted analgesic effects through coordinated regulation of nociceptive signaling pathways, addressing both peripheral and central pain mechanisms. ADSC-EVs suppress pro-inflammatory cascades by transferring miR-146a-5p and miR-223 to macrophages. This greatly inhibits factor-kappa B (NF-κB) nuclear translocation and

https://doi.org/10.2147/IJN.S598699

reducing interleukin-6 (IL-6)/TNF-α production. These inherent immunomodulatory properties suggest a potential molecular basis that could be leveraged for subsequent engineering modifications, such as surface modification of ligands targeting joint or nerve tissue, to enable more precise delivery of anti-inflammatory miRNA to the lesion site. In neuropathic pain models, these EVs attenuate spinal microglial activation by delivering heat shock protein 70 (HSP70), which inhibits NLRP3 inflammasome assembly and caspase-1-dependent IL-1β maturation.[11] Preclinical validation in rat osteoarthritis (OA) demonstrated that intra-articular administration of ADSC-EVs reduced synovial IL-6 and TNF-α levels. This effect is attributed to EV-transferred TGF-β1-mediated suppression of NF-κB signaling in macrophages.[10] Proteomic profiling revealed selective enrichment of HSP20 in ADSC-EVs, which stabilizes mitochondrial Complex IV subunits to enhance adenosine triphosphate (ATP) synthesis in dorsal root ganglion (DRG) neurons. ATP replenishment restores mitochondrial membrane potential, improving electron transport chain fidelity and reducing aberrant electron leakage, a primary source of reactive oxygen species (ROS). Consequently, HSP20-mediated metabolic rescue decreases neuronal ROS accumulation, effectively mitigating oxidative stress-induced nociceptor hyperexcitability. ADSC-EVs also exhibit significant neuroprotective and axonal regenerative capacities in pain-related pathologies. In spinal cord injury (SCI), ADSC-EVs demonstrate therapeutic efficacy for neuropathic pain and tissue repair. In a rat contusion SCI model, intrathecal administration of ADSC-EVs reduced oxidative stress in dorsal horn neurons via miR-21-5p-mediated activation of the PTEN/Akt pathway, significantly attenuating mechanical allodynia.[12] Proteomic analysis confirmed EV-mediated delivery of axonal guidance molecules Netrin-1 and Semaphorin 3A, which restored corticospinal tract integrity and improved locomotor function.[55] ADSC-EVs further rescued mitochondrial dysfunction through HSP20, increasing ATP production and reducing lesion cavity volume. These findings validate ADSC-EVs as a multimodal therapy for SCI-related pain and neural repair.

Moreover, ADSC-EVs participate in immune modulation. They shift immune responses towards an anti-inflammatory phenotype by expanding regulatory T cells (Tregs) via TGF-β1/IL-10 signaling. In RA models, EV-mediated Fas ligand (FasL) delivery induced apoptosis of activated CD68+ synovial macrophages, reducing synovial infiltration. Additionally, miR-3960 in ADSC-EVs suppressed RANKL-induced osteoclastogenesis, highlighting their dual role in immune regulation and bone protection.[13] Beyond these traits, ADSC-EVs mediate metabolic regulation. EV-encapsulated adiponectin enhances AMPK phosphorylation in skeletal muscle, alleviating insulin resistance-associated musculoskeletal pain. In type 2 diabetic models, ADSC-EVs restored mitochondrial function by delivering nicotinamide adenine dinucleotide (NADH) dehydrogenase subunits, increasing ATP synthesis and mitigating metabolic stress.[14] Engineering ADSC-EVs overexpressing long non-coding RNA (lncRNA) MALAT1 further demonstrated therapeutic potential by attenuating hypoxia-induced VEGF overexpression in RA models, inhibiting pathological angiogenesis via HIF-1α pathway interference.[56]

Overall, ADSC-EVs may exhibit more significant synergistic regulatory effects than NSAID-based pharmacotherapies. Their therapeutic advantage lies in the triple synergistic regulation of inflammation, neurological dysfunction, and metabolic disorder. The dynamic cargo composition, shaped by parental cell preconditioning, allows tailored modulation of disease-specific pathways, showcasing their tremendous edge in the field of analgesia.

Umbilical Cord MSC-Derived EVs

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) are isolated from Wharton's jelly, a non-invasive and ethically uncontroversial source. Umbilical cord mesenchymal stem cell-derived extracellular vesicles (UC-MSC-EVs) inherit a unique molecular signature from their parental cells, including surface markers such as CD9, CD63, and CD81, adhesion molecules like integrins α6β1 and CD44, and immunomodulatory proteins such as TGF-β1 and human leucocyte antigen-G (HLA-G). Their cargo is enriched with osteogenic factors like recombinant C-Type lectin domain family 11, member A (CLEC11A), anti-inflammatory miRNAs such as miR-3960 and miR-1263, and tissue-repair mediators including TSG-6 and TIMP1. Compared to EVs from other mesenchymal stem cell sources, UC-MSC-EVs exhibit enhanced stability in circulation and superior immune-modulatory potency, attributed to their unique fetal-origin molecular profile.

UC-MSC-EVs demonstrate broad therapeutic efficacy in diverse pathologies. They can mitigate cellular senescence by delivering sirtuin-activating miRNAs like miR-34a, reducing senescence-associated β-galactosidase (SA-β-gal)

activity in aged fibroblasts.[57] Besides, they can alleviate photoaging via TIMP1/Notch1.[58] For interstitial cystitis, they can alleviate neuroinflammation and mechanical allodynia in rat models by inhibiting NLRP3 inflammasome activation.[59] Moreover, UC-MSC-EVs provide a new strategy for radiation heart disease therapy.[60] They can improve radiation-induced damage of energy metabolism, ultrastructure, oxidative stress and calcium transients in cardiac organoids by regulating p53 signaling pathway, oxidative phosphorylation and copper metabolism.

UC-MSC-EVs demonstrate multimodal analgesic effects by precisely regulating nociceptive pathways, as validated across diverse preclinical pain models. Their anti-inflammatory activity is among the most thoroughly characterized aspects of their therapeutic profile. In a chronic constriction injury (CCI) rat in vitro model,[7] TGF-β-primed UC-MSC-EVs deliver elevated levels of lncRNA UCA1. This lncRNA binds and sequesters miR-96-5p, relieving its repression of FOXO3a. The resulting decrease in spinal microglial activation lowers the production of pro-inflammatory mediators such as IL-6, TNF-α, and IL-1β. Both mechanical allodynia and thermal hyperalgesia are reversed in the acute and chronic phases of pain. When administered intrathecally, UC-MSC-EVs also normalize the glutamate/γ-aminobutyric acid (GABA) balance in dorsal horn neurons, an effect that prevents central sensitization in spinal nerve ligation models.[61] UC-MSC-EVs further contribute to analgesia by shaping the immune microenvironment. In a CCI-induced rat model neuropathic pain,[7] they restore miR-181c-5p levels, which are downregulated in injured spinal cords, through targeted delivery to endothelial cells. This miRNA suppresses myeloid differentiation factor 88 (MYD88)-dependent NF-κB /NLRP3 signaling, leading to reduced secretion of IL-1β and COX-2 to alleviate neuropathic pain and neuroinflammation. Moreover, in rat osteoarthritis (OA) models,[15] intra-articular UC-MSC-EVs attenuate synovial inflammation by polarizing macrophages to an M2 phenotype. Synovial prostaglandin E2 (PGE2) levels decline, cartilage integrity improves and pain-free mobility is restored.[7] Additionally, UC-MSC-EVs help preserve joint integrity in OA-associated pain.[15] They inhibit cartilage-degrading enzymes such as matrix metalloproteinases (MMP-13) and ADAMTS-5, while promoting anti-inflammatory mediators like TSG-6. These actions maintain chondrocyte viability and reduce synovial PGE2 levels, correlating with improved pain-free mobility. UC-MSC-EVs also restore synaptic homeostasis in chronic pain states by rebalancing neuronal excitatory-inhibitory signaling. Continuous intrathecal infusion in spinal nerve ligation models[61] reduces glutamate excitotoxicity by downregulating phosphorylated N-Methyl-D-aspartic acid (NMDA) receptors and upregulating GABA-synthesizing enzymes. Proteomic analysis reveals that EV-mediated delivery of metabotropic glutamate receptor 5 (mGluR5) and potassium-chloride cotransporter 2 stabilizes chloride gradients and neuronal excitability, resolving central sensitization in pain.

In summary, UC-MSC-EVs orchestrate analgesia through four principal mechanisms: epigenetic regulation of neuroinflammation, polarization of macrophages toward an anti-inflammatory M2 phenotype, preservation of joint homeostasis as well as restoration of synaptic equilibrium. Collectively, UC-MSC-EVs may represent a transformative, cell-free approach for intractable pain management, bridging molecular specificity with clinical translatability.

Bone Marrow MSC-Derived EVs

Bone marrow mesenchymal stem cell-derived extracellular vesicles (BMSC-EVs) are nanosized membrane vesicles enriched with functional nucleic acids like mRNA, miRNA, proteins, and lipids that mediate critical intercellular communication within skeletal and systemic microenvironments. Distinct from other MSC-EVs, BMSC-EVs exhibit pronounced osteogenic programming due to their origin in bone marrow niches. They are characterized by a unique miRNA cargo that orchestrates bone formation and remodeling. They harbor a repertoire of osteogenesis-promoting miRNAs, including miR-22-3p, miR-16-5p, miR-335, and miR-29b-3p. This collectively regulate skeletal homeostasis through Wingless-Type MMTV Integration Site Family (Wnt) /β-catenin activation, phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) suppression, and NF-κB modulation.[62]

BMSC-EVs showcase broad therapeutic efficacy in diverse pathologies through tissue-specific mechanisms. In ischemic stroke (IS), BMSCs-derived small EVs antagonize cerebral endothelial Caveolin-1 driven autophagic degradation of tight-junction proteins to protect blood-brain barrier (BBB) post-stroke.[62] BMSC-EVs significantly inhibit the fibrotic process both in vitro and in vivo. This greatly reduce the fibrotic tissue generation of the shoulder capsule and improve shoulder mobility. Moreover, using exosome miRNA sequencing and knockout validation, BMSC-EVs inhibited

shoulder stiffness via the let-7a / Tgfbr1 axis.[63] The miR-181d carried in BMSC-EVs can ultimately alleviate the progression of renal fibrosis disease by downregulating the KLF 6 gene expression.

BMSC-EVs exhibit promising therapeutic mechanisms in pain management. BMSC-EVs ameliorate axon regeneration to alleviate pain. A specialized CD271+CD56+ BM-MSC subpopulation, identified via single-cell RNA sequencing, secretes exosomes that significantly enhance axonal regrowth in SCI models.[16] These EVs promote DRG neurite outgrowth in vitro and restore corticospinal tract integrity in vivo by activating the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway. At the same time, they suppress RhoA/ROCK signaling, key regulators of growth cone dynamics. Proteomic analysis reveals EV enrichment of miR-21-5p and miR-222-3p, which respectively target PTEN and SOCS3. They also derepress pro-regenerative signaling cascades.[64] BMSC-EVs also enhance tissue repair in OA-associated pain. Intra-articular EV administration in sodium iodoacetate-induced OA rats improved paw withdrawal latency (PWL) at 6 weeks post-treatment. Mechanical pain sensitivity and hyperalgesia were assessed using PWL with results demonstrating a significant improvement.[65] This is correlated with reduced articular cartilage degeneration and normalized subchondral bone remodeling.[17] Mechanistically, EV-encapsulated miR-127-3p targets cadherin-11 to block Wnt/β-catenin hyperactivation. This process further attenuates aberrant nerve invasion and angiogenesis in subchondral bone.[7] Besides, by delivering TIMP-1 and miR-140-5p, EVs inhibit cartilage-degrading enzymes and promote chondrocyte viability. Consequently, synovial PGE2 levels are reduced, and joint function is improved. Moreover, BMSC-EVs suppress pro-inflammatory cascades in joint and neural tissues. In IL-1β-stimulated OA models, EVs reduced IL-6 and TNF-α production and inhibited NLRP3 inflammasome activation. This anti-inflammatory effect is partially mediated by miR-127-3p. It suppresses NF-κB nuclear translocation and downstream COX-2/PGE2 signaling. In lumbar facet joint OA mice,[17] EV treatment normalized MMP-13 and aggrecan expression while reducing synovial IL-1β levels. This leads to sustained pain relief. Similarly, in peripheral nerve injury (PNI) models, BMSC-EVs polarized macrophages to an anti-inflammatory M2 phenotype. This reduces pro-inflammatory cytokines such as IL-1β and TNF-α and attenuates neurogenic inflammation.[66] Relevant study has indicated that high-dose bone marrow MSCs transplantation can relieve pain earlier, significantly reduce spinal IL-1β and TNF-α levels. They also boast stronger analgesic and anti-inflammatory effects than low-dose bone marrow MSC transplantation.[67] Proteomics confirm EV enrichment of Galectin-1 and PD-L1, which bind CD45 and PD-1 on T cells, suppressing TCR signaling and IFN-γ production.[67] BMSC-EVs also regulate immune responses in chronic pain. They exhibit unique immunosuppressive properties mediated by surface-bound regulatory molecules and miRNA cargo. Studies using RA-relevant in vitro models show that BMSC-EVs curtail CD4+ T cell proliferation and trigger apoptosis of activated T lymphocytes through FasL/Fas interactions.[18] Simultaneously, they appear to foster the emergence of CD4+CD25+Foxp3+ Tregs cells, an outcome linked to membrane-associated TGF-β1 and the induction of IL-10 secretion.[68] Thus, the relationship between MSC-EVs and Treg biology is not entirely settled. One report noted that while MSC-EVs left the proliferation of bulk CD3+ T cells largely unaffected, they did induce apoptosis in both CD3+ and CD4+ subsets and, paradoxically, enhanced both proliferation and apoptosis within the Treg compartment.[69] Such findings hint that MSCs and their secreted EVs may operate through overlapping but not identical immunomodulatory routes, and that the net effect on Treg populations likely hinges on experimental variables—dose, target cell activation status, and culture conditions. Sorting out these subtleties will require further study. In addition, BMSC-EVs selectively inhibit B cell and NK cell effector functions. In CpG-stimulated B cells, EVs reduce immunoglobulin secretion through miR-23b-3p targeting of BLIMP1, though B cell proliferation is minimally affected.[70] For NK cells, EV-delivered TGF-β1 downregulates NKG2D expression, impairing granzyme B release and synovial infiltration in RA.[19]

Overall, BMSC-EVs represent a multifaceted therapeutic platform for pain management and tissue regeneration. Their unique molecular cargo is shaped by the bone marrow microenvironment. This enables precise modulation of inflammation, immune responses, tissue and nerve repair. In contrast to other source of MSC-EVs, BMSC-EVs exhibit superior osteogenic and bone-targeting capacities. These traits render them particularly effective in osteoporosis and joint degeneration. The mechanisms, validated in SCI, OA, and RA models, highlight BMSC-EVs' unique capacity to address structural, inflammatory and immune-driven pain pathways. All these characteristics offer a cell-free alternative to conventional therapies.

IMSC-Derived EVs

Induced pluripotent stem cell-derived mesenchymal stem cell extracellular vesicles (IMSC-EVs) are generated through a two-step differentiation-secretion process, differentiation of iPSCs into MSCs, followed by EV secretion. These EVs inherit the regenerative and immunomodulatory properties of their parental iPSC-MSCs while avoiding ethical concerns and donor variability associated with primary MSCs. IMSC-EVs exhibit two distinct subpopulations, large EVs (LEVs) enriched in tetraspanins like CD63, CD81 and small EVs (SEVs) carrying non-coding RNAs like miR-21, miR-146a. Their molecular cargo is uniquely tailored to include dual-specificity phosphatases like DUSP2, DUSP3, TGF-β superfamily members like GDF-15, and matrix metalloproteinase inhibitors like TIMP-1. This enables precise regulation of inflammatory and tissue-repair pathways. Produced under chemically defined conditions, iMSC-EVs demonstrate enhanced stability in synovial fluid and superior batch-to-batch consistency compared to primary MSC-EVs. This ensures reproducible therapeutic outcomes.

Clinical studies have revealed broad therapeutic potential of iPSC-EVs in treating various diseases.[71] For Tendinopathy, intra-tendinous injection of iMSC-LEVs restores collagen I/III ratios and reduces glycosaminoglycan deposition greatly in collagenase-induced rat models. This enhances tenocyte migration and collagen synthesis.[72] IPSC-derived EVs are plentiful with antioxidant enzyme peroxiredoxins (PRDXs). Transfer of PRDXs by these EV attenuated cellular senescence phenotypes, such as increased SA-β-gal, p21, p53, IL-6, and γ -H2AX in both replicative and genetically induced senescent MSCs.[73] In addition, iPSC- EV enriched with miR-302b-3p exert anti-fibrotic effects, reducing inflammation and ameliorating cardiac fibrosis in vivo.[74] IMSC-EVs also promote tissue regeneration. In skin wound healing, they facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis.[75]

IMSC-EVs alleviate pain through three main interlinked mechanisms targeting neuroinflammation, anti-inflammatory response, and peripheral sensitization. IMSC-EVs reprogram macrophage phenotypes to establish an anti-inflammatory microenvironment. In tendinopathy models, LEVs polarize synovial macrophages from pro-inflammatory M1 (CD86+) to anti-inflammatory M2 (CD206+) phenotypes within 7 days.[20] Ultimately, this process synergistically inhibits NF-κB nuclear translocation and elevates IL-10 production. Central sensitization is an important process in pain development. It is characterized by spinal microglial activation and NLRP3 inflammasome-driven IL-1β overproduction. IMSC-EVs resolve neuroinflammation by silencing central sensitization pathways. In SCI models, intrathecal administration of iMSC-EVs delivers DUSP2 and DUSP3 to spinal microglia. This delivery dephosphorylates p38 mitogen-activated protein kinase (MAPK), reducing IL-1β and TNF-α release. Concurrently, EVs upregulate K^+/Cl^- cotransporters (KCC2) chloride transporters in dorsal horn neurons, restoring GABAergic inhibition and reversing hyperexcitability.[21] Besides, in peripheral sensitization, persistent inflammation or tissue injury triggers transient receptor potential vanilloid 1 (TRPV1) and voltage-gated sodium channels (Nav1.8) hyperactivation in nociceptors. This subsequently amplifies pain signals. IMSC-EVs desensitize peripheral nociceptors through targeted ion channel modulation. In OA models, EV-delivered resolvin D1 (RvD1) and glial cell line-derived neurotrophic factor (GDNF) downregulate TRPV1 and ASIC3 expression in sensory nerve endings. Consequently, this process reduces mechanical allodynia.[22] Systemic analgesia manifest via decreased serum neuropeptides like calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), highlighting broad-spectrum efficacy.[21]

Hence, iMSC-EVs represent a promising paradigm shift in precision pain therapy, combining the scalability of iPSC technology with the safety of cell-free interventions. Their unique mechanisms span across neuroimmune crosstalk regulation, anti-inflammatory reprogramming, and nociceptor desensitization. This addresses both peripheral and central pain pathways, as validated in tendinopathy, OA, and neuropathic pain models. IMSC-EVs offer unparalleled batch consistency and molecular customization. This mechanism enables tailored modulation of disease-specific targets like DUSP2/3-p38 axis in microglia, TRPV1 in sensory neurons. They also boast tremendous potential in tissue repair and regeneration. Further research should prioritize clinical validation and scalable manufacturing to unlock their full potential for intractable pain syndromes.

A Source-Comparative Framework for EV Function in Pain

The preceding subsections have catalogued the molecular cargo and therapeutic actions of EVs from four distinct stem cell compartments. When these profiles are laid side by side, a pattern of functional divergence begins to take shape—one

that carries real implications for how one might select among these vesicles for a given pain indication. OA offers a useful lens through which to examine this divergence, not least because all four EV types have been tested in preclinical OA models. ADSC-EVs blunt synovial inflammation by delivering TGF-β1, which suppresses NF-κB signaling in macrophages and drives down IL-6 and TNF-α production.[10] UC-MSC-EVs pursue a related but mechanistically distinct path. miR-1208 shuttled by these vesicles steers macrophages toward an M2 phenotype, lowering PGE2 levels and helping to preserve cartilage structure.[15] BMSC-EVs intervene at a different anatomical site altogether. Through transfer of miR-127-3p, they silence cadherin-11 and dampen aberrant Wnt/β-catenin activity in subchondral bone, an effect that not only relieves pain but also checks the structural remodeling that fuels progressive joint deterioration.[17] iMSC-EVs add yet another layer of complexity. Rather than engaging primarily with immune cells or bone, they carry RvD1 and GDNF to sensory nerve endings, where they downregulate TRPV1 and ASIC3 and directly quiet peripheral nociceptors.[22] The therapeutic logic thus diverges sharply. ADSC-EVs and UC-MSC-EVs operate chiefly at the synovial immune interface, BMSC-EVs target the osteochondral junction, and iMSC-EVs act on the neuronal hardware of pain itself.

This kind of functional specialization extends well beyond OA. In models of SCI, ADSC-EVs work through a dual mechanism. MiR-21-5p activates the PTEN/Akt axis to relieve oxidative stress in dorsal horn neurons, while guidance molecules like Netrin-1 and Semaphorin 3A help rebuild damaged corticospinal tracts.[12] BMSC-EVs also promote axonal regrowth, but they do so by tipping the balance between PI3K/Akt/mTOR and RhoA/ROCK signaling in favor of growth cone advancement.[16] UC-MSC-EVs, examined in the context of chronic constriction injury, tackle the problem from a different angle entirely. Their cargo includes lncRNA UCA1, which sponges miR-96-5p and relieves repression of FOXO3a, ultimately dialing down spinal microglial activation.[7] IMSC-EVs operate further downstream in the sensitization cascade. They deliver DUSP2 and DUSP3 to dephosphorylate p38 MAPK in microglia while simultaneously upregulating KCC2 to restore chloride homeostasis and GABAergic inhibition.[21]

Immunological behavior offers another axis for comparison, and here the differences are equally instructive. In RA models, ADSC-EVs clear activated synovial macrophages through FasL-mediated apoptosis and put a brake on osteoclastogenesis via miR-3960-directed suppression of RANKL signaling.[13] BMSC-EVs cast a wider net: they inhibit CD4+ T cell proliferation, expand Treg populations through TGF-β1/IL-10 signaling, and restrain the effector functions of both B cells and NK cells.[18] This broader immunological footprint likely reflects the hematopoietic milieu of the bone marrow niche. UC-MSC-EVs, by contrast, appear to concentrate their immunomodulatory efforts more narrowly on macrophage polarization and inflammasome restraint. This profile is consistent with the immune-privileged status of their fetal tissue of origin.[7]

Metabolic regulation reveals further distinctions. ADSC-EVs stand out for their capacity to address the metabolic drivers of pain. They deliver adiponectin to enhance AMPK phosphorylation in skeletal muscle and supply NADH dehydrogenase subunits that help salvage mitochondrial ATP production in diabetic settings.[14] Neither UC-MSC-EVs nor BMSC-EVs share this pronounced metabolic orientation; their payloads lean more heavily toward immunomodulation and osteogenesis, respectively. IMSC-EVs, produced under chemically defined conditions, offer a different sort of advantage. Their superior batch consistency and scalability speak to translational feasibility rather than mechanistic novelty.[71]

Taken together, it becomes clear that stem cell-derived EVs are not interchangeable commodities. Their therapeutic emphasis, be it metabolic rescue, immune reprogramming, structural repair, or direct neuromodulation, is imprinted by the tissue microenvironment of their parental cells. This source-dependent identity supplies a rational basis for matching EV types to pain etiologies. When metabolic dysfunction dominates the clinical picture, as in diabetic neuropathy, ADSC-EVs may align most closely with the underlying pathophysiology. For pain rooted in structural compromise of joints or nerves, the osteogenic and axonal repair repertoire of BMSC-EVs appears uniquely relevant. In settings of widespread immune dysregulation, the broader suppressive capacity of BMSC-EVs—or the more focused macrophage-polarizing effects of UC-MSC-EVs—may hold greater appeal. Such a framework, drawn from direct comparison of the available preclinical evidence, may inform both the selection of native vesicles for therapeutic development and the rational design of engineered variants that deliberately combine features from multiple cellular origins.

Nervous System-Derived EVs

Neuron-Derived EVs

Neuron-derived extracellular vesicles (NDEVs) are enriched with functional biomolecules including synaptic proteins like SNAP25, L1CAM, neuroactive miRNAs like miR-124, miR-146, and pathological cargo such as β-amyloid (Aβ) and phosphorylated Tau (p-Tau). Their release responds dynamically to three key stimuli, neuronal activity, oxidative stress, and inflammatory signals like TNF-α and IL-6. NDEVs exhibit neuron-specific molecular signatures distinct from other EVs. These include surface adhesion markers like integrins α6β1, CD44 and synaptic regulators, reflecting their central nervous system (CNS) origin. While L1CAM has traditionally been used for NDEV isolation, recent studies emphasize the need for neuron-specific markers like synaptophysin to improve purification specificity.[76] NDEVs transport unique miRNA subsets like miR-146a-5p, miR-21-5p and immunomodulatory proteins like FasL, TGF-β1. This cargo can enable three core functions, synaptic plasticity modulation, neuroinflammatory regulation, and pathological protein propagation. These mechanisms underpin NDEVs' dual roles in neurodegeneration and pain pathways, as detailed in subsequent sections.

NDEVs play dual roles in neurodegenerative diseases, acting as both pathological propagators and potential therapeutic vehicles. In Alzheimer's disease (AD), NDEVs transport toxic Aβ oligomers and p-Tau. This can accelerate amyloid plaque formation and neuroinflammation. Simultaneously, they also deliver protective miRNAs like miR-185 that suppress amyloid-β precursor protein (APP) expression to mitigate Aβ generation.[23] In amyotrophic lateral sclerosis (ALS), NDEVs spread misfolded superoxide dismutase (SOD1) and TDP-43 aggregates to astrocytes and microglia, driving motor neuron degeneration. Their engineering forms can silence disease-associated miRNAs like miR-24-3p to restore neuroplasticity.[77] In PD, α-synuclein-laden NDEVs propagate neuronal toxicity but can also be harnessed to deliver neuroprotective miRNAs like miR-7 that inhibit α-synuclein (α-syn) aggregation.[78] This dual cargo-shuttling ability highlights their therapeutic potential. CRISPR-edited NDEVs can block disease factors, while miRNA-loaded NDEVs may enhance protection.

NDEVs orchestrate pain persistence through distinct mechanisms in the central nervous system, peripheral nerves, and neuro-immune interface. In the central nervous system, NDEVs remodel synaptic plasticity to encode pain states. They transfer miR-132 to the spinal cord, which targets Ephexin5, thereby relieving its suppression of GluN2B-containing NMDA receptors.[79] This disinhibition enhances NMDA receptor-mediated postsynaptic currents, promoting central sensitization and consolidating a"pain memor". Complementarily, NDEV-borne BDNF activates presynaptic and postsynaptic TrkB receptors, engaging the ERK/CREB signaling cascade. This pathway upregulates the transcription of pain-facilitating neuropeptides, including CGRP and Substance P (SP), thereby sustaining hyperalgesia. Additionally, NDEVs contribute to neuroinflammatory cascades by shuttling cytokines such as TNF-α and IL-6 to spinal microglia.[24] These vesicles activate microglial TLR/MyD88 pathways, triggering NLRP3 inflammasome assembly and IL-1β release, which further exacerbates central sensitization. In peripheral sensory pathways, NDEVs directly modulate mitochondrial function, neuronal metabolism, and nociceptive signaling. During diabetic peripheral neuropathy (DPN), hyperglycemia-stressed neurons release NDEVs loaded with oxidized mitochondrial DNA.[25] Upon delivery to recipient sensory neurons, this DNA activates the ROS/JNK pathway, triggering excessive mitochondrial fission and bioenergetic deficits (ATP depletion), which drive spontaneous nociceptor firing.[23] Concurrently, miR-21 packaged within NDEVs suppresses PTEN expression in peripheral neurons, leading to amplified PI3K/Akt signaling and TRPV1 channel hyperactivation. This process augments peripheral nociceptive inputs and contributes to pain hypersensitivity.[24] Conversely, NDEVs derived from healthy neurons deliver protective cargo, such as NADH dehydrogenase subunits and HSP70, to compromised neurons. This transfer restores electron transport chain integrity, rescues oxidative phosphorylation, and alleviates pain by mitigating metabolic stress. At the neuro-immune interface, NDEVs function as bidirectional communicators to fine-tune inflammatory pain. In OA models, neuronal-derived vesicles deliver miR-146a-5p to synovial macrophages. This miRNA suppresses NF-κB activation, leading to reduced production of IL-1β and PGE2, and promotes the expansion of Tregs.[10] Concurrently, Fas ligand present on the NDEV surface engages Fas receptors on activated T cells, inducing apoptosis and curtailing autoimmune-driven pain pathways. Collectively, these spatially and mechanistically segregated actions establish NDEVs as master regulators of pain pathogenesis, integrating synaptic, metabolic, and immunomodulatory mechanisms to adapt to nociceptive stimuli.

Broadly speaking, NDEVs act as pivotal mediators of neuron-microenvironment crosstalk. They exhibit dual roles in neurodegeneration and pain regulation. Pathologically, NDEVs propagate misfolded proteins and pro-inflammatory signals, accelerating disease progression. Conversely, they deliver neuroprotective molecules that maintain neural homeostasis. Within pain pathways, NDEVs crucially modulate three key processes, neuroinflammation suppression, synaptic plasticity adaptation, and metabolic reprogramming. This multifaceted modulation establishes NDEVs as novel targets for non-opioid analgesic development.

Microglia-Derived EVs

Microglia-derived extracellular vesicles (MDEVs) dynamically mirror microglial activation states, which shift between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes under pathological stimuli. MDEVs carry signature cargo including microglia-specific surface markers like CD11b, Iba1, immunomodulatory proteins like TNF-α, IL-1β, neurodegenerative disease-associated cargo like α-syn oligomers, Aβ aggregates, and regulatory miRNAs like miR-146a-5p. Their release responds precisely to inflammatory triggers like lipopolysaccharide (LPS), IFN-γ and protein aggregates like Aβ, α-syn. Crucially, disease-stage remodeling of lipidomic components like cholesterol, polyunsaturated fatty acids and proteomic profiles like TREM2, P2RY12 establishes MDEVs as functional state biomarkers. The functional dichotomy of MDEVs is intrinsically linked to microglial polarization dynamics, M1-Polarized MDEVs and M2-Polarized MDEVs. Under pro-inflammatory conditions, neurotoxic M1-MDEVs deliver IL-6, TNF-α, Aβ oligomers, and p-Tau to recipient cells. These cargo components activate NLRP3 inflammasomes in astrocytes while suppressing mitophagy, thereby impairing mitochondrial quality control. This cascade ultimately exacerbates neuronal damage and synaptic loss. Neuroprotective M2-MDEVs transport IL-10, TGF-β, TREM2, and miR-124. Such mediators enhance Aβ clearance through lysosomal degradation pathways and restore bioenergetics via NAD+ salvage activation. STAT6-dependent signaling simultaneously dampens glial activation, collectively preserving CNS homeostasis.[80] This plasticity enables MDEVs to function as either pathological vectors or therapeutic vehicles.

MDEVs orchestrate disease-specific mechanisms in neurodegenerative and neuropsychiatric disorders through context-dependent cargo sorting. In AD, MDEVs propagate Aβ and p-Tau through non-synaptic routes, accelerating plaque deposition. Conversely, TREM2-enriched variants promote phagocytic clearance and restore neuronal mitochondria.[81] PD's pathology involves MDEV-mediated transfer of α-synuclein oligomers, which nucleate Lewy bodies. IFN-γ-primed vesicles further sustain inflammation through MHC-II/TNF-α delivery and NLRP3 activation.[82] For ALS, MDEVs spread mutant SOD1 and miR-155, damaging motor neuron mitochondria and amplifying neuroinflammation.[77] Additionally, MDEVs also show diagnostic value, with Vitamin D-binding protein serving as a depression biomarker.[83] MiR-146a-5p secreted by microglia can also act as a key signaling mediator during neurogenesis in depression, positioning miR-146a-5p as a therapeutic target in depression.[84] Furthermore, post-ischemic MDEVs deliver IL-1β and miR-155 to penumbral regions during IS.[85] This cargo exacerbates BBB disruption through MMP-9 upregulation and microvascular thrombosis.

MDEVs play multifaceted roles in pain modulation by dynamically interacting with neural and immune systems. In neuropathic pain models, MDEVs derived from activated microglia modulate inflammatory signaling and cellular stress responses. For instance, M1-polarized MDEVs carry pro-inflammatory cytokines such as TNF-α and IL-1β, which can influence neuronal excitability and promote nociceptive sensitization.[86] Furthermore, beyond their role in neuronal sensitization, MDEVs exert critical immunomodulatory effects that contribute to the maintenance of cellular homeostasis in the nervous system. Research has indicated that MDEVs activate autophagy and regulate the expression of key genes involved in neuroinflammation and apoptosis, such as caspase-1, caspase-8, and IL-1β, thereby modulating microglial homeostasis and potentially influencing pain-related signaling pathways.[87] Concurrently, spinal microglia-derived MDEVs deliver NLRP3 inflammasome components, specifically ASC and caspase-1, to dorsal horn neurons. This delivery promotes IL-1β maturation while enhancing excitatory postsynaptic currents, ultimately sustaining central sensitization and chronic pain states.[88] This inherent "signal transduction" capability suggests that MDEVs can be engineered to transform from disease-causing vectors into therapeutic vectors by loading anti-inflammatory miRNAs like miR-146a or IL-1 receptor antagonists. Beyond inflammation, MDEVs critically influence synaptic plasticity and pain memory through molecular and structural remodeling. Chronic pain is associated with aberrant synaptic pruning

mediated by complement proteins such as C1q. MDEVs actively transport C1q to hyperactive synapses, facilitating their tagging for microglial phagocytosis. Although essential for neural circuit refinement, dysregulated pruning disrupts pain pathway homeostasis and perpetuates maladaptive plasticity. Epigenetic mechanisms further consolidate pain memory. In traumatic brain injury (TBI) model, miR-155-enriched MDEVs suppress SOCS1 expression in spinal glia. This suppression amplifies JAK/STAT3 signaling and increases glial-derived neurotrophic factor release such as GDNF.[27] Consequently, synaptic strength escalates, and pain-related neural circuits are stabilized.[89] Additionally, MDEVs exhibit neuroprotective and regenerative capacities that counterbalance their pro-nociceptive effects. M2-like MDEVs deliver functional mitochondrial DNA and NADH dehydrogenase subunits to damaged neurons. This cargo restores oxidative phosphorylation while reducing ROS accumulation, thus alleviating nociceptor hyperexcitability. Furthermore, these vesicles promote axonal regeneration by transporting guidance molecules Netrin-1 and Semaphorin 3A. Such molecules activate PI3K/Akt/mTOR pathways to stimulate DRG neurite outgrowth, facilitating functional recovery after SCI.[28] Notably, MDEVs maintain myelin homeostasis and remyelination—processes indirectly resolving pain.[90] In demyelinating conditions like chemotherapy-induced neuropathy, M2-polarized MDEVs transfer pro-remyelination factors like miR-219, CNTF to oligodendrocyte precursor cells (OPCs). This transfer drives OPC differentiation into mature myelinating oligodendrocytes,[26] restoring saltatory conduction while reducing ectopic firing in pain pathways. Conversely, chronic inflammation shifts microglial EVs toward an M1 phenotype that exacerbates demyelination through IL-1β and ROS overproduction, ultimately entrenching neuropathic pain. Lastly, MDEVs also orchestrate immune-neural crosstalk to reshape pain. By delivering miR-124 to peripheral macrophages, these vesicles suppress TLR4 signaling and polarize macrophages toward an anti-inflammatory M2 phenotype. Consequently, IL-6 and PGE2 production decrease, thereby mitigating inflammatory pain. Concurrently, PD-L1 displayed on MDEV surfaces engages PD-1 receptors on T cells. This interaction inhibits TCR signaling and IFN-γ secretion, attenuating autoimmune-mediated pain.[91] Collectively, these immunomodulatory actions demonstrate the functional plasticity of MDEVs. Their cargo composition and microglial activation states determine whether they perpetuate or resolve pathological pain.

Hence, MDEVs function as indispensable mediators in neurodegenerative diseases and chronic pain. They simultaneously act as pathological vectors propagating toxic proteins like Aβ, α-syn and regenerative agents delivering protective molecules like TREM2, miR-124, demonstrating significant therapeutic potential. These vesicles may modulate three key pain-regulatory dimensions, neuroinflammatory signaling, synaptic plasticity dynamics, and immune-neural crosstalk. Consequently, MDEVs provide promising targets for developing complementary non-opioid analgesics.

Astrocyte-Derived EVs

Astrocyte-derived extracellular vesicles (ADEVs) inherit a unique molecular profile from their parental astrocytes, astrocytes, the most abundant glial cells in the CNS. They include astrocyte-specific markers like glial fibrillary acidic protein, metabolic regulators like lactate transporters, and disease-associated cargo such as Aβ, p-Tau, and α-syn. Their secretion dynamically increases in response to pathological stimuli such as Aβ/α-syn aggregates and inflammatory cytokines such as IL-6, TNF-α. Notably, ADEVs exhibit bidirectional roles in CNS homeostasis. Under physiological conditions, they support neuroplasticity and BBB integrity by transferring neurotrophic factors like BDNF and synaptic proteins. Paradoxically, in disease states, they propagate neurotoxic proteins and inflammatory mediators, accelerating neurodegeneration.

ADEVs exhibit context-dependent roles in neurological disorders, ranging from propagating pathology to enabling repair. In AD, ADEVs exacerbate Aβ and p-Tau spread by packaging these aggregates alongside neurotoxic factors such as BACE1, complement proteins C3b/C5b-C9. Paradoxically, ultrasound-stimulated ADEV release may transiently mitigate Aβ toxicity.[92] In PD, astrocytes expressing mutant leucine-rich repeat kinase 2 (LRRK2) secrete ADEVs deficient in miR-200a-3p. This deficiency fails to suppress JNK-mediated dopaminergic neuron degeneration. Concurrently, α-synuclein-loaded ADEVs may indirectly accelerate disease progression.[93] In ALS, ADEVs deliver mutant SOD1 and SEMA3A-activating signals due to miR-494-3p loss. These actions drive motor neuron degeneration while enhancing IL-6-mediated neuroinflammation.[77]

ADEVs exhibit dual regulatory roles in pain modulation through their influence on neuroinflammation and synaptic plasticity. Under chronic pain conditions, activated astrocytes release ADEVs containing pro-inflammatory cytokines and

specific miRNAs such as miR-34a. These miRNAs suppress neurotrophic signaling by targeting proteins such as NTRK3 and Bcl2.[94] This suppression contributes to reduced dendritic complexity, impaired synaptic stability, and heightened neuronal hyperexcitability, which collectively drive central sensitization in neuropathic pain models. A pivotal mechanism in this process is the ADEV-facilitated sustainment of neuroinflammation. They shuttle chemokines like fractalkine (CX3CL1) to pain-processing regions, recruiting immune cells and perpetuating a chronic inflammatory state. Complementing this, activated astrocytes themselves release ATP. ATP then activates neuronal P2X/P2Y purinergic receptors, inducing mechanical allodynia and thermal hyperalgesia, a finding corroborated by optogenetic studies showing that spinal astrocyte activation triggers ATP release.[95] This release upregulates MAPK phosphorylation and amplifies IL-1β production.[96]

Conversely, ADEVs derived from astrocytes in a reparative state or subjected to protective preconditioning can promote analgesia and neural repair. For instance, anti-inflammatory stimulus-derived ADEVs deliver miR-146a that inhibits NF-κB signaling in bone cancer pain, reducing pro-inflammatory cytokine release.[30] Beyond pain modulation, ADEVs facilitate neural repair through targeted molecular delivery. Beyond pain modulation, these vesicles facilitate neural repair. In SCI and stroke models, hypoxia-preconditioned ADEVs enriched with miR-21 suppress Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling in astrocytes.[29] This phenotypic shift reduces glial scar formation. Furthermore, ADEVs contribute to remyelination and neuronal recovery by delivering neurotrophic factors like BDNF, GDNF activate both PI3K/Akt and ERK pathways in neurons.[97] These activated pathways stimulate axonal regeneration and synaptic reorganization. Evidence also suggests that lncRNA in ADEVs also demonstrated strong potential of neural recovery. LncRNA released from ADEVs modulate microglial phenotype after TBI. Besides, they maintain the integrity of BBB and increase the density of neurons and myelinating cells, thereby facilitating the recovery of neural structures via SMAD7 regulation.[98] By balancing inflammatory responses with structural repair mechanisms, ADEVs establish themselves as versatile mediators in pain pathology and recovery processes.

In conclusion, ADEVs serve as dynamic mediators of astrocyte-neuron communication. They intricately regulate pain pathways and neural repair through context-dependent cargo delivery. In pain transmission, ADEVs either exacerbate or attenuate signaling via synaptic modifications and inflammatory cascades. Conversely, during neural repair, they drive axonal regeneration and glial cell phenotypic reprogramming. Future research should prioritize cargo engineering, stimulus-specific modulation, and delivery optimization. These advances may unlock ADEVs' full therapeutic potential for precision pain management.

Oligodendrocyte-Derived EVs

Oligodendrocyte-derived extracellular vesicles (ODEVs) own ample myelin-associated proteins such as myelin oligodendrocyte glycoprotein, 2′,3′-cyclic nucleotide 3′-phosphodiesterase, metabolic regulators like monocarboxylate transporters, and neuroprotective cargo such as SOD, catalase, and BDNF. ODEVs are released in response to neuronal activity or stress signals like hypoxia, oxidative stress. Their secretion is tightly regulated by axonal demand, enabling dynamic support for axonal integrity, energy metabolism, and stress resistance. A hallmark feature of ODEVs is their bidirectional communication with neurons. They are internalized by axons via endocytosis, delivering critical biomolecules that maintain axonal transport, mitochondrial function, and synaptic stability.

ODEVs play context-dependent roles across neurological disorders, balancing pathological dissemination and therapeutic repair. In PD, they propagate α-synuclein aggregates while concurrently delivering antioxidant enzymes to mitigate oxidative neuronal damage.[77] For AD, ODEVs predominantly serve neuroprotective roles by enhancing Aβ clearance and neutralizing ROS. However, chronic stress may redirect their cargo toward inflammatory mediators.[99] Following cerebral ischemia, ODEVs facilitate post-stroke repair through anti-inflammatory and pro-regenerative mechanisms. They suppress astrogliosis by delivering miR-219a-3p to astrocytes, inhibiting NF-κB-driven production of pro-inflammatory cytokines.[100] Simultaneously, ODEVs enhance remyelination through lipid/protein transfer, restoring axonal conduction velocity.[101]

ODEVs regulate pain pathogenesis through multimodal mechanisms, including neuroinflammatory resolution, axonal metabolic support, and myelin repair. ODEVs mitigate pain-associated neuroinflammation through targeted delivery of anti-inflammatory and antioxidant cargo. In chronic neuropathic pain models, ODEVs are internalized by spinal

microglia. These vesicles transfer miR-146a-5p, silencing TLR4 signaling and suppressing NLRP3 inflammasome activation. This reduces IL-1β and IL-18 release, attenuating central sensitization.[102] Additionally, in peripheral sensory pathways, ODEV-derived SOD and catalase neutralize ROS in DRG neurons. This action prevents oxidative stress-induced neuronal hyperexcitability and mechanical allodynia. What's more, ODEVs are critical for maintaining axonal integrity in pain pathways. In the central nervous system, ODEVs provide crucial metabolic and bioenergetic support to maintain axonal integrity and function. They achieve this by delivering key cargo such as the deacetylase SIRT2, which enhances mitochondrial efficiency and ATP production in axons. This transcellular transfer of SIRT2 via ODEVs is essential for sustaining axonal energy homeostasis.[103] Additionally, ODEVs contribute to synaptic modulation by transporting factors like BDNF and miR-132 to spinal dorsal horn terminals, where they help regulate synaptic plasticity and glutamatergic transmission.[104] They also support axonal energy metabolism by supplying monocarboxylate transporters (MCTs), which facilitate lactate uptake and help sustain local ATP production in metabolically challenged axons.[32] In central post-stroke pain, ODEVs counteract axonal atrophy by replenishing neurofilament proteins. At the same time, they stabilize microtubule networks via RhoA/ROCK pathway modulation.[31] Beyond anti-inflammation and axonal support, ODEVs also facilitate remyelination in demyelination-associated pain conditions. In multiple sclerosis (MS), they transfer cholesterol and proteolipid protein (PLP) to oligodendrocyte precursor cells (OPCs), promoting their differentiation into mature myelinating oligodendrocytes.[105] This process restores saltatory conduction while reducing ectopic firing in nociceptive pathways. It ultimately alleviates neuropathic pain. In vivo studies show that ODEV administration in cuprizone-induced demyelination models enhances myelin regeneration and reverses thermal hyperalgesia.[32]

In a nutshell, ODEVs serve as versatile mediators of CNS homeostasis, balancing roles in pathology propagation and neuroprotection. In neurodegenerative diseases like PD and AD, ODEVs exhibit context-dependent duality. They disseminate toxic aggregates like α-synuclein yet paradoxically deliver antioxidant defenses. For pain regulation, ODEVs suppress neuroinflammation, preserve axonal integrity and promote remyelination. Thus, this may offer potential avenues for non-opioid analgesic strategies to us.

Schwann Cell-Derived EVs

Schwann cells (SCs) are the myelinating glia of the peripheral nervous system (PNS). Schwann cell-derived extracellular vesicles (SC-EVs) contain abundant SC-specific markers such as myelin protein zero, CD63, neurotrophic factors like BDNF, GDNF, and regulatory miRNAs such as miR-21, miR-142-5p. SC-EVs are released in response to nerve injury, metabolic stress, or neuronal activity. Their biogenesis is dynamically regulated by SC phenotypic states. Repair SCs activated after injury secrete exosomes distinct from those of differentiated SCs like mature myelinating cells. Repair SC-EVs are characterized by elevated levels of c-Jun and Sox2 transcription factors, which drive their regenerative cargo composition. SC-EVs are internalized by axons and neurons via endocytosis, facilitating bidirectional communication critical for axonal maintenance, metabolic support, and injury response.

SC-EVs have demonstrated therapeutic potential across diverse conditions through targeted molecular delivery and phenotypic modulation. In optic nerve injury, SC-EVs enhance optic nerve regeneration by activating pro-regenerative signaling in retinal ganglion cells (RGCs). Specifically, repair SC-derived EVs transfer miR-21 to suppress PTEN expression, triggering PI3K/Akt/mTOR pathway activation that drives axonal elongation and growth cone formation. Concurrently, SC-EVs deliver RNA-stabilizing proteins like ELAVL4 / HuD, preserving synaptic mRNA integrity and reducing RGC apoptosis post-injury.[106] For PNI, SC-EVs accelerate functional recovery after sciatic nerve crush or transection. They promote axonal regrowth via miR-21-mediated PI3K/Akt activation and PTEN inhibition in DRG neurons. These vesicles also transfer cytoskeletal regulators to stabilize dendritic spines. This dual action enables structural and functional reintegration of regenerated axons.[107] In DPN, SC-EVs exhibit dual roles shaped by metabolic stress.[35] Hyperglycemia alters EV cargo, elevating miR-142-5p levels that target ACTN4 and ELAVL4 in hippocampal neurons. This greatly impairs synaptic plasticity and exacerbates cognitive-pain comorbidities.[33] Conversely, engineering SC-EVs overexpressing miR-21 restore neurite outgrowth under high glucose by reactivating PI3K/Akt pathways and mitigating oxidative damage. All these mentioned above highlight their therapeutic adaptability.[108]

SC-EVs regulate neuroinflammatory processes in chronic pain states. After PNI, SC-EVs at the lesion site gain access to the systemic circulation. These vesicles are then passively distributed throughout the body. Their subsequent entry into the hippocampus is facilitated by a neuroinflammatory state that increases the permeability of the blood-brain barrier, allowing circulating EVs to preferentially extravasate into and accumulate within this limbic structure. Research demonstrates that under such stress, SC-EVs exhibit a marked decrease in anti-inflammatory miRNAs cargo like miR-146a-5p. The loss of this miRNA fuels neuroinflammation by shifting macrophages polarization from an anti-inflammatory (pro-M2) to a pro-inflammatory (pro-M1) phenotype via the TRAF6/NF-κB pathway.[109] When these reprogrammed SC-EVs deliver their content to hippocampal cells, they directly transmit a pro-inflammatory signal from the peripheral nerve injury site to the central nervous system, thereby establishing a direct mechanistic link between PNI and hippocampal neuroinflammation in chronic neuropathic pain states. In a CCI rat model, miR-142-5p suppresses ACTN4 and ELAVL4, destabilizing actin filaments and mRNA stability in dendritic spines. These molecular disruptions correlate with memory deficits and persistent pain hypersensitivity.[110] Conversely, repair SC-derived EVs deliver anti-inflammatory miR-21 to spinal microglia. This cargo inhibits NLRP3 inflammasome activation, consequently reducing IL-1β and TNF-α production. These actions finally attenuate neuropathic pain through neuroimmune suppression. Besides, SC-EVs are critical for maintaining axonal integrity and synaptic function in pain pathways. By transferring MCTs, SC-EVs supply lactate to metabolically stressed axons, sustaining ATP production and preventing axonal degeneration in the peripheral nervous system.[111] In SCI, SC-EVs counteract axonal atrophy through dual mechanisms. They replenish neurofilament proteins to enhance cytoskeletal stability while activating the PI3K/Akt/mTOR/p70S6K pathway. This coordinated signaling cascade drives axonal regrowth by promoting protein synthesis and microtubule assembly.[34] Furthermore, SC-EVs drive neural recovery in neuropathic pain models through dual regenerative mechanisms. On one hand, they enhance axonal regeneration by transferring miR-21 and growth factors to injured neurons. This cargo reactivates PI3K/Akt signaling and suppresses caspase-3-dependent apoptosis.[112] On the other hand, SC-EVs derived from skin precursor SCs or repair SCs promote remyelination by delivering cholesterol and PLP to SCs. This restores saltatory conduction and reduces ectopic firing in nociceptive pathways. Preclinical studies confirm that SC-EVs administration rescues motor and sensory function in TBI models.[34]

In brief, SC-EVs are multifaceted mediators of peripheral and central nervous system repair. Their cargo composition and cellular origin determine whether they exacerbate or alleviate pathological pain. In optic nerve injury, peripheral neuropathy, and diabetic complications, SC-EVs enhance axonal regeneration, suppress apoptosis, and stabilize synaptic integrity through miRNA- and protein-dependent mechanisms. Their ability to modulate neuroinflammation and axonal metabolism positions SC-EVs as promising therapeutic agents for chronic pain.

Comparative Functions of NDEVs Across Pain-Relevant Pathologies

The five EV populations surveyed in this section all originate within the nervous system, yet their functional contributions to pain diverge in ways that become most apparent when examined side by side in shared pathological settings. A comparative look across several disease contexts reveals both the distinct signature of each EV type and the points at which their actions intersect.

SCI offers a useful starting point, as it draws on both neuroprotective and structural repair mechanisms. M2-polarized MDEVs deliver guidance molecules, Netrin-1 and Semaphorin 3A, that activate PI3K/Akt/mTOR signaling in damaged axons and stimulate neurite outgrowth.[28] ADEVs address a different obstacle altogether. When derived from hypoxia-preconditioned astrocytes, they carry miR-21 and suppress JAK2/STAT3 signaling in recipient astrocytes, thereby limiting the glial scar that physically impedes regeneration.[29] SC-EVs tackle axonal repair from yet another angle: they replenish neurofilament proteins to stabilize the cytoskeleton while engaging PI3K/Akt/mTOR/p70S6K signaling to drive protein synthesis and microtubule assembly.[34] The division of labor is telling. MDEVs provide directional cues, ADEVs clear the path by reducing scar burden, and SC-EVs supply the trophic and structural resources needed for axon extension. NDEVs and ODEVs appear less prominently in the SCI studies reviewed here, hinting that certain EV types may be preferentially recruited by specific forms of neural injury.

DPN shifts the lens toward metabolic stress and its consequences for EV cargo. Under hyperglycemic conditions, NDEVs package oxidized mitochondrial DNA and transfer it to sensory neurons, where it triggers ROS/JNK signaling,

excessive mitochondrial fission, and ATP depletion—events that culminate in spontaneous nociceptor firing.[25] SC-EVs undergo a parallel but distinct reprogramming. Their miR-142-5p content rises, targeting ACTN4 and ELAVL4 in hippocampal neurons and destabilizing dendritic spines in brain regions tied to pain processing and cognition.[33] The two vesicle populations thus contribute to DPN pathology through complementary routes: NDEVs amplify nociceptive drive locally within the peripheral sensory system, whereas SC-EVs transmit the impact of peripheral nerve stress to central circuits, forging a link between neuropathy and its cognitive-affective sequelae. This central relay function appears to be a specialized role of SC-EVs within the neural EV network.

Demyelinating disorders highlight a different axis of specialization, one centered on myelin maintenance. ODEVs dominate this space. They supply SIRT2 to axons, boosting mitochondrial efficiency and sustaining ATP production under metabolic strain.[103] They also provide monocarboxylate transporters to facilitate lactate uptake, offering an alternative fuel source to compromised fibers.[32] In multiple sclerosis models, ODEVs transfer cholesterol and proteolipid protein to oligodendrocyte precursor cells, driving their maturation and restoring saltatory conduction.[105] MDEVs engage the same problem from an immunological direction. M2-polarized MDEVs carry pro-remyelination factors like miR-219 and CNTF to OPCs,[26] while chronic inflammation can tip the balance toward M1-MDEVs that worsen demyelination through IL-1β and ROS.[90] SC-EVs extend this repair logic to the periphery, delivering cholesterol and proteolipid protein to Schwann cells and reducing ectopic firing in nociceptive pathways after traumatic brain injury.[34] The pattern that emerges is straightforward: ODEVs act as primary custodians of central myelin, MDEVs modulate the inflammatory backdrop that governs remyelination efficiency, and SC-EVs perform analogous functions in peripheral nerves.

Neuropathic pain brings the interplay between inflammation and synaptic plasticity into sharper focus. M1-polarized MDEVs deliver NLRP3 inflammasome components to dorsal horn neurons, promoting IL-1β maturation and boosting excitatory postsynaptic currents that sustain central sensitization.[87] NDEVs operate directly on the synaptic machinery. They transfer miR-132 to spinal neurons, relieving Ephexin5-mediated suppression of GluN2B-containing NMDA receptors and strengthening the connections that encode persistent pain.[79] NDEV-derived BDNF further activates TrkB receptors and the ERK/CREB cascade, driving transcription of CGRP and substance P.[24] The two vesicle types converge on central sensitization through different doors: MDEVs stoke the neuroinflammatory fire that lowers synaptic thresholds, while NDEVs reinforce the very architecture of hyperexcitable synapses. In the periphery, SC-EVs add another dimension. After nerve injury, SC-EVs from the lesion site enter the circulation and accumulate in the hippocampus, where a drop in anti-inflammatory miR-146a-5p tilts macrophage polarization toward an M1 phenotype via TRAF6/NF-κB signaling.[109] This relay translates local nerve damage into central inflammatory and behavioral changes.

Stepping back from the details, a few organizational principles stand out. Each neural EV population leans toward a particular pathological niche. ODEVs and SC-EVs in axonal and myelin support, MDEVs in neuroimmune regulation, NDEVs in synaptic tuning, and ADEVs in scar modulation and barrier integrity. Disease states can flip EV cargo from protective to pathogenic, a switch most vividly illustrated by the M1/M2 dichotomy of MDEVs and the metabolic reprogramming of NDEVs and SC-EVs in diabetes. A spatial logic also runs through the network: SC-EVs handle peripheral-to-central communication, ODEVs and ADEVs operate largely within central compartments, and MDEVs and NDEVs work at the synapse-immune interface.

These comparisons carry practical weight. For conditions driven by central sensitization, NDEVs or M2-MDEVs may offer the most direct mechanistic alignment. Where demyelination predominates, ODEVs or SC-EVs become the more logical candidates. The network perspective also urges restraint: suppressing a pathogenic EV population. For example, M1-MDEVs without regard for timing or localization risks undercutting the protective efforts that other EVs in the same network are trying to mount.

Bacterial EVs

Bacterial extracellular vesicles (BEVs) encapsulate proteins, nucleic acids, lipids, and metabolites from their parental cells. The biogenesis of BEVs differs between gram-negative and gram-positive bacteria. Gram-negative bacteria produce outer membrane vesicles (OMVs) through controlled blebbing of the outer membrane, carrying LPS, periplasmic proteins, and enzymes. In contrast, Gram-positive bacteria generate cytoplasmic membrane vesicles (CMVs)

enriched with peptidoglycan (PG). These vesicles respond to environmental stressors, such as antibiotics, and mediate interbacterial competition.[113] For example, they can deliver antimicrobial toxins to eliminate rival bacteria. Their stability allows for systemic distribution across biological barriers, reaching distant organs like the brain and heart to influence host physiology.

BEVs exhibit context-dependent roles across diseases, mediated by their cargo and the "gut-organ axis" networks they modulate. Gut microbiota-derived extracellular vesicles (GMEVs), a kind of BEVs, directly modulate brain homeostasis by traversing the BBB. In AD, Escherichia coli OMVs deliver β-amyloid and pro-inflammatory miRNAs to neurons. This process exacerbates neuroinflammation and synaptic loss.[114] Conversely, probiotic BEVs exhibit neuroprotective properties. Lactobacillus paracasei-derived BEVs reduce hippocampal neuroinflammation and restore cognitive functions in hyperammonemic rats by suppressing NLRP3 inflammasome activation.[115] This duality underscores BEVs as bidirectional modulators of the gut-brain axis, where dysbiosis-induced BEVs propagate pathology, while probiotic vesicles confer neuroprotection. The gut-brain axis is a dynamic, bidirectional communication network linking the normal intestinal flora, GMEVs and the CNS. It plays a crucial role in regulating normal and abnormal biological processes like neurodegenerative diseases. On one hand, the gut microbiome (GM) consists of a variety of microbial communities that interact with the host through many processes, such as the production of bioactive compounds and GMEVs. These GMEVs carry miRNAs, proteins, and other bioactive substances as mediators that facilitate communication between GM and CNS. On the other hand, CNS can alter the composition and function of GM through the autonomic nervous system and neuroendocrine pathways. This affects the microbial reproduction, metabolism, and the release of GMEVs.[116] Similar regulatory patterns are evident in cardiovascular disease. In atherosclerosis, Porphyromonas gingivalis OMVs degrade vascular integrity and promote plaque instability. In contrast, commensal Akkermansia muciniphila produces BEVs containing anti-inflammatory miR-30c, which attenuates endothelial inflammation and may stabilize plaques.[117] The gut-bone axis reveals additional therapeutic dimensions. Probiotic Lactobacillus BEVs mitigate glucocorticoid-induced osteonecrosis by enhancing osteogenic activity and promoting angiogenesis.[52] This demonstrates their capacity for targeted tissue remodeling through systemic communication. In IS, commensal-derived BEVs influence recovery via immunomodulation. Bacteroides fragilis vesicles polarize microglia toward anti-inflammatory M2 phenotypes through TLR2/STAT3 signaling, significantly reducing cerebral infarct volume and improving neurological outcomes.[118]

BEVs modulate pain through neuroinflammation, axonal support, and neural repair, bridging gut-brain-bone axis communications. Pathobiont-derived BEVs exacerbate pain by activating nociceptors and glial cells, with Gram-negative BEVs enriched in LPS and Gram-positive BEVs carrying PG, driving systemic inflammation. LPS increases intestinal permeability "leaky gut", enters circulation, and activates joint tissue macrophages via TLR4 priming, the first hit in OA pathogenesis. This sensitizes joints to damage-associated molecular patterns (DAMPs) that trigger pro-inflammatory cascades, correlating with OA radiographic severity. Similarly, in RA, PG activates synovial fibroblasts through TLR/NOD receptors, upregulating matrix metalloproteinase (MMPs) and cytokines.[38] Concurrently, dysbiotic microbiota BEVs transport miR-155 to spinal microglia, amplifying NF-κB-driven TNF-α/IL-1β release that potentiates central sensitization.[119] These inflammatory mediators potentiate central sensitization and chronic neuropathic pain. In contrast, commensal BEVs exert protective effects. They promote axonal integrity via cargo-mediated metabolic and cytoskeletal regulation. Lactobacillus plantarum CMVs deliver miR-132 and ELAVL4 RNA-binding proteins to neurons. These components stabilize synaptic mRNAs such as β-actin and GAP-43, consequently enhancing dendritic spine density. In chemotherapy-induced peripheral neuropathy (CIPN), Faecalibacterium prausnitzii BEVs supply MCTs, shuttling lactate to metabolically stressed axons. This lactate flux maintains ATP production, ultimately preventing axonal degeneration.[36] Additionally, BEVs naturally coordinate multi-organ communication through the gut-bone-brain axis. The gut-bone-brain axis provides a mechanistic framework for how osteonecrosis, a localized skeletal disorder, can propagate neuroinflammation in distant brain regions like the hippocampus. Osteonecrosis establishes a persistent inflammatory microenvironment within bone, characterized by ischemia, cell death, and the release of damage-associated molecular patterns (DAMPs). This fuels a state of chronic systemic inflammation, with elevated levels of pro-inflammatory cytokines circulating throughout the body. These circulating mediators compromise blood-brain barrier integrity and activate hippocampal microglia. Once activated, microglia release their own suite of inflammatory factors like IL-1β, TNF-α,

initiating a neuroinflammatory cascade that impairs synaptic plasticity and neuronal function in the hippocampus, a region critical for mood and cognition. This process directly links the peripheral skeletal pathology to central nervous system dysregulation, exacerbating the pain experience. BEVs derived from Lactobacillus animalis act as multi-target therapeutic agents. By homing to the osteonecrotic site and stimulating bone repair through enhanced angiogenesis and osteogenesis, they eliminate the primary source of systemic inflammation.[37] The consequent reduction in peripheral inflammatory signals de-activates the hippocampal microglial response, thereby breaking the gut-bone-brain axis circuit and alleviating associated neuropathic pain.[120]

Ultimately, BEVs are versatile nanoplatforms that mediate critical inter-organ communications via the gut-brain, gut-bone, and gut-heart axes. In diseases ranging from Alzheimer's to osteonecrosis, BEVs deliver pathogenic or protective cargo to distant tissues, influencing neuroinflammation, axonal stability and tissue repair. These vesicles exhibit context-dependent duality in pain. They exacerbate nociception through immune activation yet resolve it via neurotrophic support. Engineering BEVs may offer remarkable opportunities for multi-axis therapies, combining scalability, biocompatibility, and precise targeting. Future research should address cargo standardization, in vivo tracking, and clinical translation. Hopefully, BEVs may be harnessed as next-generation nanomedicines for intractable diseases and pain syndromes.

Macrophage-Derived EVs

Macrophage-derived EVs (MDEVs) switch between two polarization state, pro-inflammatory M1 and anti-inflammatory M2. These vesicles encapsulate distinct cargo profiles. M1-MDEVs are enriched with pro-inflammatory mediators and damage-associated molecular patterns like DAMPs. M2-MDEVs carry anti-inflammatory factors, tissue-repair miRNAs such as miR-146a, miR-223, and metabolites like glutamate. Release is dynamically regulated by microenvironmental cues. Nutrient stress enhances glycolytic enzyme packaging, while hypoxia promotes mitochondrial DNA inclusion.[121] MDEVs communicate with target cells via receptor binding TLR4 or endocytosis, enabling precise modulation of immune responses and tissue homeostasis.

MDEVs orchestrate disease pathogenesis through immunometabolism reprogramming and context-dependent cargo sorting. MDEVs regulate bone metabolism in aging by dual cargo delivery. M2-MDEVs deliver glutamate to inhibit osteoclast NFATc1 signaling, suppressing bone resorption.[122] Inflammatory osteoclast-derived MDEVs enhance mineralization via LINC02381-stabilized osteria transcription. Osteoporotic nutrient stress reprograms MDEV cargo, reducing osteogenic miR-324. Simultaneously, this increases sclerostin-mediated Wnt inhibition to accelerate bone loss.[123] Besides, MDEVs exhibit dual roles in vascular inflammation as validated in Atherosclerosis.[124] M1-MDEVs propagate plaque instability by transferring miR-155 to endothelial cells, which disrupts tight junctions and promotes monocyte adhesion. These vesicles also deliver inflammasome components to vascular smooth muscle cells. Consequently, this drives IL-1β secretion and necrotic core expansion. Conversely, M2-MDEVs attenuate disease via miR-146a-mediated suppression of TRAF6/NF-κB signaling, reducing inflammatory cytokine production in preclinical models.[125] In Acute Respiratory Distress Syndrome, MDEVs regulate the dynamic shift from lung injury to repair. During the exudative phase, M1-MDEVs exacerbate alveolar damage by shutting mitochondrial DAMPs to epithelial cells.[126] This process induces ferroptosis through GPX4 suppression. They also recruit neutrophils via CXCL1 chemokines. As inflammation resolves, M2-MDEVs dominate. They clear apoptotic cells through phosphatidylserine exposure and secrete TGF-β1 to promote fibroblast-mediated tissue repair. Metabolic rewiring during recovery increases MDEV packaging of adenosine, which dampens neutrophil activation.

MDEVs regulate pain pathogenesis through immunomodulation and neural repair coordination. MDEVs resolve chronic pain by targeting neuroinflammatory cascades in the CNS. M2-MDEVs deliver TGF-β1 and miR-146a to microglia. This process consequently silences TLR4/MyD88 signaling and reduces NLRP3 inflammasome assembly. This cascade inhibits IL-1β maturation and TNF-α release, attenuating central sensitization.[127] The anti-inflammatory cargo repolarizes microglia from pro-inflammatory M1 to immunosuppressive M2 phenotypes. This further disrupts sustained neuroimmune activation in chronic pain states.[128] Concurrently, MDEVs provide metabolic and structural support to compromised sensory neurons. M2-MDEVs supply functional mitochondria to metabolically stressed DRG neurons. They rescue oxidative phosphorylation and prevent ATP depletion-induced axonal degeneration. This process

requires CD200R receptor-ligand coordination between macrophages CD200R+ and sensory neurons iSec1+ to enable targeted organelle delivery.[129] They also deliver essential molecules, such as growth factors, miRNAs, and matrix-modulating enzymes, that enhance cellular energy metabolism, promote extracellular matrix (ECM) remodeling, and facilitate tissue regeneration, thereby aiding the recovery of functionally impaired cells in diabetic wounds.[39] Moreover, MDEVs promote axonal repair. M2-MDEVs deliver Wnt5a to Schwann cells, activating β-catenin pathways that enhance remyelination of damaged nociceptive fibers. In CNS trauma, miR-219-mediated ROCK inhibition stabilizes microtubule networks to reverse axonal transport deficits.[40]

Hence, MDEVs represent dynamic mediators of disease progression and resolution, with polarization status dictating their functional impact. Their ability to simultaneously regulate neuroinflammation and tissue repair positions MDEVs as promising therapeutic vectors.

Tumor-Derived EVs

Tumor-derived extracellular vesicles (TDEVs) encapsulate oncogenic cargo including DNA, RNA, proteins, lipids, and metabolites reflective of their parental tumor. The release of TDEVs is amplified by hypoxia, acidosis, and inflammatory stimuli within the tumor microenvironment (TME). TDEVs exhibit dual functional identities. On one hand, they serve as pathogenic carriers. They are enriched with MMPs, thrombin-sensitive proteins, and immunosuppressive molecules like PD-L1. On the other hand, they function as intercellular communicators. Their surface-adhered integrins like α6β4, αvβ5 enable organotropic targeting, facilitating pre-metastatic niche formation. The stability in circulation and capacity to cross biological barriers underpin systemic effects on distal organs.

TDEVs serve as double-edged tools in oncology, driving disease progression yet offering diagnostic and therapeutic opportunities. Their miRNA/lncRNA profiles like miR-21-5p provide tumor-specific biomarkers for early detection and metastasis prediction.[130] For instance, breast cancer-derived TDEVs transporting miR-105 disrupt BBB integrity by degrading tight junction protein ZO-1.[131] Beyond oncology, TDEV levels of HSP90α correlate with intestinal ischemia severity.[132] Hence, TDEVs provides prognostic value for tissue recovery outcomes and cancer diagnosis. TDEVs also orchestrate cancer progression through multifaceted pathobiological programs. They deliver HSP70 and MMP-2 to induce epithelial-mesenchymal transition and increase vascular permeability, thereby establishing pre-metastatic niches in distant organs.[133] Concurrently, TDEVs reshape the TME through immune reprogramming.[42] They mediate multiple immunosuppressive mechanisms. PD-L1$^+$ TDEVs bind PD-1 receptors on cytotoxic T lymphocytes, inhibiting anti-tumor immunity. CD73$^+$ TDEVs generate adenosine to suppress T-cell clonal expansion via A2AR signaling. During immune cell modulation, TDEVs promote M2 macrophage polarization. At the same time, they suppress NK cell cytotoxicity and upregulate neutrophil PD-L1. Additionally, they transform fibroblasts into cancer-associated fibroblasts, further supporting tumor growth.[134] Mechanistically, TDEVs execute these functions by regulating NF-κB, JAK-STAT, and PI3K/Akt/mTOR pathways.[135] These pathways drive inflammatory responses and metabolic reprogramming that may collectively establish an immunosuppressive TME.

TDEVs coordinate cancer pain pathogenesis by synergistically amplifying neuroinflammatory cascades and driving maladaptive neural plasticity within pain-processing circuits. TDEVs directly activate glial-immune crosstalk to sustain neuroinflammation in cancer pain. Upon uptake by spinal microglia, TDEV-delivered miR-155 suppresses SOCS1, amplifying TLR4/NF-κB signaling and NLRP3 inflammasome assembly. This drives IL-1β and TNF-α release, potentiating central sensitization.[41] Concurrently, TDEVs transfer MMP-1 to astrocytes, activating protease-activated receptor 1 (PAR1) and enhancing neuronal responsiveness to nociceptive stimuli. TDEVs induce maladaptive plasticity in sensory pathways by disrupting cytoskeletal integrity and receptor signaling. Bioinformatics analyses confirm TDEV miRNAs target critical neural maintenance pathways. They destabilize actin cytoskeleton through F-actin network disassembly in DRG neurons, impairing structural stability. Besides, they guide impaired axon via plexin-A2 receptor downregulation, which disrupts semaphorin-mediated repulsive signaling. By suppressing p75 neurotrophin receptor-dependent TrkB recycling, they also promote neuronal hyperexcitability. In early stage cancer, TDEV uptake by sensory neurons induces de novo sensitization through PAR2/F2RL1 activation.[130] This process generates spontaneous pain prior to overt tissue damage.

In conclusion, TDEVs exemplify the intricate coupling of cancer progression and pain pathogenesis. TDEVs sculpt the tumor microenvironment, drive neural remodeling, and amplify nociceptive signaling through parallel neuroimmune and cytoskeletal mechanisms. Their dual roles as metastatic facilitators and pain initiators highlight TDEVs as master regulators of chronic cancer pain. Targeting TDEV biogenesis, cargo, or receptor interactions offers promising avenues to disrupt the pain-metastasis axis. Advancing TDEV-based diagnostics and therapeutics necessitates deeper mechanistic insights into their spatiotemporal actions within neural networks.

Plant-Derived EVs

Plant-derived extracellular vesicles (PDEVs) have garnered much attention, but it is important to emphasize that their biological effects are highly specific to their plant source. Different PDEVs exhibit unique advantages such as abundant source, low cost, low immunogenicity, and high biocompatibility. They also possess intrinsic, source-dependent biological characteristics like antioxidant, anti-inflammatory, and regenerative properties.[136]

The therapeutic efficacy of PDEVs is mediated by plant-specific cargo. For instance, Platycodon grandiflorum-derived EVs (PGEVs) reprogram the immune microenvironment and show significant anti-tumor effects in triple-negative breast cancer by inducing ROS and polarizing macrophages to the M1 phenotype.[137] In contrast, Ginseng-derived nanoparticles alleviate inflammatory bowel diseases (IBD) via the p62/Nrf2/Keap1 pathways.[43] Simultaneously, they also inhibit TLR4/MAPK-mediated pro-inflammatory signaling like ZO-1, occludin.[138] This dual action restores epithelial barrier integrity via upregulation of tight junction proteins. Similarly, ginger exosome-like nanoparticle delivers miRNA therapeutics. They inhibit intestinal inflammation through targeted suppression of PI3K/Akt signaling.[139] Furthermore, specific PDEVs, such as folic acid-modified ginger EVs, can remodel the immune microenvironment in RA via the PI3K-AKT pathway.[44] Concomitantly, this process also effectively addresses the RA-associated chronic pain. Mechanistically, PDEVs suppress PI3K/AKT-driven NLRP3 inflammasome activation in synovial macrophages and reduce pro-nociceptive IL-1β production. Concurrently, PDEV cargo directly desensitizes TRPV1 sensory nerve endings. This multi-targeted intervention reverses mechanical allodynia and thermal hyperalgesia. It demonstrates the capacity of specific PDEVs to correct immune dysregulation and nociceptive pathology in chronic inflammatory states.

Therefore, PDEVs exemplify nature-inspired nanomedicine, whose capabilities like modulating cancer progression and neuroimmune pain pathways are defined by their botanical origin. Their core strengths, oral stability, biocompatibility, and multi-organ targeting, enable potential therapeutic strategies for refractory chronic conditions. These advantages may shed light on the subsequent development of "oral-engineering plant-derived EVs" tailored for specific diseases, such as intestinal-related pain.

Serum-Derived EVs

Serum-derived extracellular vesicles (SEVs) are abundant in cell-specific biomolecules reflective of physiological and pathological states. SEVs cargo signatures serve as sensitive indicators for early disease detection and progression tracking. These diseases include brain atrophy,[140] IS and ovarian cancer.[141] Notably, SEVs elicit transient mechanical analgesia in inflammatory pain models. Research has indicated that the CD206 + macrophages were increased in the spinal cord after the SEV injection into the recipient mice.[45] The increase in the basal mechanical threshold in recipient mice was blocked by naltrexone. Thus, SEVs can induce short-term mechanical anti-pain in recipient mice. The presence of leucine enkephalin as a carrier material for the SEVs suggests that transient mechanical pain resistance is mediated by opioid signaling. SEVs derived from mouse sera helped to relieve the inflammatory pain in the recipient mice. This confirms the role of SEV in the resolution of inflammatory pain. What's more, other studies have illustrated that intrathecal injection of serum can inhibit glial responses and improve nerve conduction after CIPN.[46] The mechanism is that serum contain high levels of anti-inflammatory mediators and exosomes which are largely key mediators of analgesia.

EVs in the Pathogenesis of Pain: A Mechanistic Synthesis Across Pain Classifications

Building upon the functional profiles of EVs from diverse sources detailed in Biological Characteristics of EVs: Source-Specific Functional Profiling, this section provides a synthesized analysis from the perspective of pain pathology. Focus is shifted from listing individual EV sources to dissecting the core pathogenic drivers of different pain classifications and elucidating how the EV system, through coordinated and complementary actions, interventions in these processes. A summary of these EV-mediated mechanisms across pain classifications is presented in Table 1.

Nociceptive Pain

Nociceptive pain results from tissue damage or inflammation, characterized by a well-localized pain sensation. It manifests in two distinct forms, somatic nociceptive pain and visceral nociceptive pain.[3] Somatic nociceptive pain affects cutaneous, musculoskeletal, or articular structures. Visceral nociceptive pain arises from damage of internal thoracoabdominal organs. Clinically significant examples include acute post-surgical pain, arthritis pain like OA, injury-related pain and IBD. Its pathogenesis is dominated by the inflammatory cascade, where immune cells release a storm of mediators that directly activate and sensitize peripheral nociceptors. This peripheral drive can lead to central sensitization, amplifying pain signals within the spinal cord. EVs counter this process primarily through source-level anti-inflammatory and peripheral desensitization strategies.

Inflammation and pain are pathophysiologically intertwined through a cascade of molecular, cellular, and neural interactions. Upon tissue injury or infection, immune cells release pro-inflammatory mediators, including bradykinin, prostaglandins, cytokines like TNF-α, IL-1β, IL-6, and nerve growth factor (NGF). These mediators directly activate and sensitize peripheral nociceptors, particularly TRPV1 and Nav1.8/1.7/1.9 to depolarize sensory neurons and heighten transduction sensitivity. Concomitantly, endothelial cells exhibit increased permeability and express adhesion molecules, ICAM-1/VCAM-1. This process enables leukocyte infiltration, amplifying mediator synthesis. Within the dorsal horn, microglial activation triggered by TLR4 signaling releases BDNF and IL-6, disrupting inhibitory GABAergic transmission. Subsequently, this initiates NMDA receptor responses in second-order neurons. The process, called central sensitization, manifests as allodynia, hyperalgesia, and spontaneous pain through altered synaptic efficacy and expanded receptive fields. Furthermore, sustained inflammation induces transcriptional reprogramming in sensory ganglia via NF-κB-dependent upregulation of pain-related ion channels and neuropeptides like SP/CGRP. This establishes enduring neuroplasticity that underlies chronic pain transitions. The magnitude and duration of inflammation directly correlate with pain intensity. Consequently, unresolved inflammation drives epigenetic reprogramming and neuroimmune exhaustion. Subsequently, chronic pain ensues. In essence, inflammation converts physiological nociception into pathological pain by rewiring peripheral and central neural circuits. This mechanistic synergy underscores why inflammatory conditions invariably manifest pain and why resolving inflammation remains central to pain management.

The most prominent anti-inflammatory mechanism involves EVs reprogramming immune cells at the injury site. As detailed in Stem Cell-Derived EVs, MSC-EVs like ADSC-EVs and UC-MSC-EVs and specific PDEVs deliver anti-inflammatory miRNAs like miR-146a-5p, miR-223 to macrophages. This cargo suppresses the NF-κB pathway and NLRP3 inflammasome activation, polarizing macrophages toward an anti-inflammatory M2 phenotype and drastically reducing the levels of key pro-inflammatory cytokines like IL-6 and TNF-α. Concurrently, to directly quench neuronal hyperexcitability, EVs like those from iMSC-EVs deliver resolvins and growth factors like GDNF to sensory nerve endings, downregulating the expression of sensitized ion channels such as TRPV1 and ASIC3, thereby reversing mechanical allodynia (IMSC-Derived EVs).

In visceral inflammation, as seen in IBD, this EV-mediated immunomodulation is critical. As a kind of chronic visceral nociception pain, abdominal pain is one of IBD's common symptoms. UC-MSC-EVs mitigate pain by enhancing IL-10 expression and suppressing inducible nitric oxide synthase (iNOS), reducing inflammation that sensitizes local nociceptors.[61] They further disrupt pain signaling by inhibiting NF-κB and mTOR pathways,[61] downregulating pain-promoting neuropeptides and reducing TRPV1 trafficking.[142] Collectively, these mechanisms underpin UC-MSC-EVs' role in regulating visceral nociception pain. Conversely, BEVs from a dysbiotic gut microbiota can exacerbate pain by

critically disrupting host metabolic and signaling homeostasis (Bacterial EVs). A key mechanism involves BEVs skewing the kynurenine pathway balance away from the analgesic metabolite kynurenic acid (KynA) and towards pro-nociceptive Kyn. The accumulated Kyn promotes the release of pro-inflammatory cytokines that sensitize intestinal nociceptors.[143] The concomitant loss of KynA diminishes its inherent antagonism on NMDA and TRPV1/CGRP pathways, thereby amplifying neuroinflammation and pain signaling.[144] Thus, BEVs act as critical microbial messengers, tipping the kynurenine pathway balance away from pain-suppressive KynA towards pain-inducing metabolites like Kyn. These mechanisms directly link gut dysbiosis to neuronal hyperexcitability and cytokine-mediated nociception in the inflamed intestine.[145] Simultaneously, GMEVs emerge as a direct communication channel in the gut-brain axis, fundamentally modulating nociceptive signaling. These vesicles serve as neuroactive carriers, shuttling microbially synthesized neurotransmitters, most notably GABA, across the intestinal barrier to engage with peripheral neurons. The delivery of GABA via GMEVs to postsynaptic neurons in the enteric nervous system triggers potassium efflux, hyperpolarizing neuronal membranes and potently dampening pain signal propagation. The critical role of this pathway is evidenced by studies showing that a decrease in GABA-producing Ligilactobacillus murinus reduces fecal GABA levels and promotes visceral hypersensitivity, an effect reversible by GABA-generating probiotics.[117] Thus, GMEVs transduce the composition of the gut microbiota into either an analgesic or algesic state by directly regulating neuronal excitability. This establishes a quantifiable and rapid signaling route within the gut-brain axis that bypasses traditional endocrine pathways. The functional dichotomy of EVs, as either amplifiers or resolvers of nociception, is further exemplified by other sources. For instance, M1-MDEVs deliver TLR4 ligands to activate enteric glia and release CCL2. CCL2 sensitizes vagal afferents and increases NMDAR phosphorylation in nucleus tractus solitarius. Simultaneously, they transfer miR-155 to inhibit μ-opioid receptors on dorsal horn neurons. Thus, this process blunts endogenous analgesia. Besides, M2-MDEVs deliver Wnt5a to Schwann cells, activating β-catenin pathways that enhance remyelination of damaged nociceptive fibers (Macrophage-Derived EVs).

In brief, this mechanistic framework positions EVs as essential intermediaries in nociceptive pain, converting local tissue injury into amplified neural signaling through discrete molecular pathways. Their source-specific cargo profiles offer precision targets for next-generation analgesics.

Neuropathic Pain

Neuropathic pain is a debilitating condition arising from a lesion or disease of the somatosensory system including peripheral fibres like Aβ, Aδ and C fibres and central neurons.[146] According to their origin, examples of neuropathic pain can be classified into two categories, peripheral neuropathic pain and central neuropathic pain.[146] Peripheral neuropathic pain includes trigeminal neuralgia, PNI and DPN. Central neuropathic pain models contain SCI, brain injury, post-stroke pain and complex regional pain syndrome (CRPS). Emerging evidence implicates EVs as critical mediators in the initiation, propagation, and maintenance of neuropathic pain through multifaceted mechanisms. This pain type manifests as hypersensitivity states mediated by pathological neural excitability and neuroimmune interactions. EVs address this multifaceted challenge through a multi-targeted reparative approach, concurrently tackling structural damage, dysfunctional signaling, and immune dysregulation.

A central therapeutic strategy is the resolution of neuroinflammation and central sensitization. For instance, iMSC-EVs deliver dual-specificity phosphatases (DUSP2/3) to spinal microglia, dephosphorylating p38 MAPK and suppressing the NLRP3 inflammasome. This action thereby curtails the release of IL-1β and TNF-α, key drivers of central sensitization (IMSC-Derived EVs). Complementing this, several EV types work to restore the compromised inhibitory tone in the spinal cord.[21] UC-MSC-EVs and iMSC-EVs upregulate the KCC2 chloride transporter in dorsal horn neurons, re-establishing GABAergic inhibition and breaking the cycle of neuronal hyperexcitability (Umbilical Cord MSC-Derived EVs and 2.1.4).[147] Concurrently, a separate regenerative program is activated to repair structural damage. BMSC-EVs and SC-EVs promote axonal regeneration by activating the PI3K/Akt/mTOR pathway[16] while inhibiting the growth cone collapse mediator RhoA/ROCK.[64] This process further facilitates the regrowth of damaged fibers (Bone Marrow MSC-Derived EVs and 2.2.5).

DPN, one of the most common complications of diabetes mellitus, is caused by damaged distal sensory nerves. It clinically manifests as sensory and motor symptoms, including limb numbness, pain, tingling, hyperalgesia, etc.[148] DPN

exemplifies how metabolic distress and neural damage converge, with EVs from diverse sources acting as co-conspirators in a complex pathological network. Under persistent hyperglycemic stress, EVs undergo a pathological reprogramming that drives DPN through complementary mechanisms. SC-EVs, for instance, carry elevated levels of miR-142-5p, which impairs synaptic plasticity in the hippocampus by targeting ACTN4 and ELAVL4,[35] thereby exacerbating the pain-cognitive comorbidities frequently observed in DPN patients.[107] In a parallel and synergistic manner, NDEVs function as amplifiers of metabolic crisis. They deliver cargo such as oxidized mitochondrial DNA to recipient neurons, thus activating the ROS/JNK pathway. This cascade promotes pathological mitochondrial fission, depletes cellular ATP, and ultimately induces aberrant nociceptor firing. These processes directly convert systemic metabolic stress into neuronal hyperexcitability.[23] The intricate EV network, however, also harbors therapeutic potential. In a contrasting and restorative role, therapeutic EVs like M2-MDEVs can deliver enzymes like GAD65 to convert excitatory glutamate to inhibitory GABA, thereby rebalancing spinal neurotransmission and suppressing pain signals.[39] Thus, in DPN, SC-EVs and NDEVs collectively establish a self-reinforcing vicious cycle of pathology, while therapeutic EVs like M2-MDEVs engage in the same network to restore homeostasis. This dynamic interplay underscores the dual nature of EVs in DPN, functioning as both central orchestrators of the disease's complex pathophysiology and as promising treatments for its resolution.

The versatile role of EVs in neuropathic pain is further highlighted across diverse etiologies, from autoimmune-driven CRPS to structural SCI and cancer. In CRPS, a disorder characterized by systemic dysregulation, SEVs from patients exhibit distinct miRNA signatures with miR-21-3p and miR-146a/b showing incredible elevation.[149] These miRNAs modulate innate immune hyperactivation via TLR4/MyD88 signaling. Strikingly, these EV miRNA profiles can differentiate treatment responders, positioning them as promising predictive biomarkers,[150] while MDEVs reduced thermal hyperalgesia through IL-10-dependent mechanisms.

In the context of SCI, a concerted EV-mediated reparative program can be engaged. ADSC-EVs execute a dual-pathway strategy. They mitigate oxidative stress in dorsal horn neurons by delivering miR-21-5p to activate the PTEN/Akt pathway. This process leads to reduction of ROS and suppression of neuronal hyperexcitability.[12] Concurrently, they restore axonal integrity by transporting guidance molecules to rebuild damaged corticospinal tracts.[55] This reparative function is complemented by UC-MSC-EVs, which directly target neuroinflammatory pathways through epigenetic regulation. In CCI rat models of neuropathic pain, these EVs carry lncRNA UCA1, which sequesters miR-96-5p to relieve its repression of FOXO3a, thereby suppressing spinal microgliosis and normalizing the synaptic glutamate/GABA balance[7] (Umbilical Cord MSC-Derived EVs). Besides, under neuropathic conditions, macrophages release EVs containing miR-155. After sensory neurons in the dorsal root ganglia take up these EVs, their IL-6 expression is upregulated. This indicates that these EVs may mediate the transmission of inflammatory signals from macrophages to neurons, thereby augmenting neuropathic pain.[151]

Chronic cancer-related pain is defined as pain caused primary by the cancer itself or by its treatment. It is listed as a sort of chronic secondary pain syndromes and includes neuropathic and musculoskeletal pains.[152] The role of EVs in chronic cancer-related pain extends beyond TDEVs to involve a synergistic network of vesicles from multiple cellular sources. TDEVs initiate peripheral sensitization by delivering specific cargo, such as MMP-1 and thrombin-sensitive protein 1, which activate PAR1/PAR2 on nociceptors[42] (Tumor-Derived EVs). This initial signal is significantly amplified into a self-sustaining cycle through the recruitment of other EV populations. Research has proved that MMPs 1 and thrombin-sensitive protein 1 expression increase in TDEVs.[153] Ultimately, this persistent peripheral drive engages the central nervous system, where ADEVs and MDEVs solidify the pain state by mediating central sensitization and synaptic plasticity. Viewing cancer pain through the lens of this multi-EV collaborative network provides a more comprehensive framework for understanding its complexity and for developing targeted interventions that disrupt this pathological cell-cell communication at multiple levels. Hopefully, early targeted intervention of cancer will be accomplished, and new insights will be provided for multi EV-based analgesia.

In conclusion, neuropathic pain stems from somatosensory system damage, with EVs critically mediating its pathogenesis and resolution through multifaceted mechanisms. Pathogenic EVs orchestrate neuropathic pain by transporting dysregulated molecular cargo that amplifies neuroinflammation and metabolic stress. Hence, they drive maladaptive neuro-immune interactions and neuronal hyperexcitability. Conversely, therapeutic EVs counter these processes

by coordinately suppressing neuroimmune hyperactivation, restoring axonal integrity, and rebalancing excitatory-inhibitory neurotransmission. Thus, across the spectrum of neuropathic pain, EVs form a complex communication network. All these mechanisms collectively reverse aberrant neural plasticity. Clinically, they function as predictive biomarkers reflecting pain progression and as engineering delivery platforms for precision neuromodulation. This dual functionality positions EVs as pivotal agents bridging mechanistic insights to targeted interventions across neuropathic pain etiologies.

Nociplastic Pain

Nociplastic pain originates from altered nociception process without identifiable tissue injury or somatosensory system pathology. This condition manifests as heightened pain responsiveness mediated by maladaptive central neural plasticity. The symptoms observed in nociplastic pain include multifocal pain that is more widespread or intense than the other two types.[154] The mechanisms underlying this sort of pain remain incompletely understood. However, it is recognized that augmented CNS pain and sensory processing and altered pain modulation play prominent roles.[155] Nociplastic conditions often have high co-prevalence rates with each other and with other chronic pain conditions. This kind of pain can be exemplified by fibromyalgia, irritable bowel syndrome (IBS) and tension-type headache. What distinguishes nociplastic pain from a mechanistic standpoint is the primacy of central sensitization in the absence of ongoing peripheral pathology. In nociceptive pain, central sensitization is typically driven by sustained peripheral input; in neuropathic pain, by injury to the somatosensory system itself. In nociplastic states, however, the central amplification machinery appears to operate with a degree of autonomy, maintained by alterations in glial function, synaptic connectivity, and descending modulatory tone rather than by continuous peripheral drive. EVs participate in this process at multiple levels, as the following discussion illustrated.

Fibromyalgia is a prevalent pain syndrome characterized by widespread pain in the absence of evident tissue injury or pathology, rendering it one of the most mysterious chronic pain conditions. BEVs drive fibromyalgia's nociplastic pain by transducing gut dysbiosis into maladaptive brain-gut axis signaling, establishing self-sustaining central sensitization (Bacterial EVs). Upon breaching the intestinal barrier, dysbiosis-derived BEVs activate two parallel pathways that together drive central sensitization. On one hand, they ascend nociception through vagal afferent stimulation. In this way, BEV cargo excites enteric neurons projecting to the nucleus tractus solitarius, thus amplifying thalamocortical pain processing. On the other hand, they initiate neuroimmune crosstalk through peripheral monocyte activation and spinal microglial engagement. This subsequently triggers IL-17-dependent synaptic potentiation in dorsal horn circuits. The central sensitization manifests clinically as widespread mechanical allodynia and diffuse pain, a hallmark of nociplastic pathology. The net effect is a self-sustaining cycle of central hyperexcitability. BEVs also undermine the endogenous analgesic systems that would normally keep such amplification in check. They deplete analgesic metabolites—bile acids and GABA among them—and thereby weaken descending inhibitory control from the rostral ventromedial medulla. Concurrently, BEV-mediated suppression of colonic farnesoid X receptor (FXR) signaling dysregulates hypothalamic-pituitary-adrenal axis function and reduces prefrontal cortical μ-opioid receptor expression. This constellation of effects —central sensitization, impaired descending inhibition, and neuroendocrine dysregulation—creates the conditions for persistent, widespread pain that no longer requires ongoing peripheral input to sustain itself.[156] Interventions like vagal nerve stimulation or FXR agonists demonstrate therapeutic efficacy by disrupting this brain-gut dialogue. This kind of intervention may confirm BEVs as regulators of fibromyalgia's pain vulnerability signature.

Ulcerative colitis (UC), a kind of chronic IBD, is an immune-mediated disease affecting the colonic mucosa. The typical symptoms of ulcerative colitis are abdominal pain, diarrhea due to inflammation of the colonic mucosa. The researchers revealed the critical role of BEVs in UC (Bacterial EVs). BEVs drive nociplastic pain in ulcerative colitis through interconnected gut-brain axis pathways. In UC patients, IgA-coated BEVs accumulate in inflamed colonic tissue and activate TLR4 signaling on mucosal immune cells.[157] This triggers local release of pro-inflammatory cytokines that directly sensitize intestinal nociceptors. They manifest clinically as chronic abdominal pain. Upon entering systemic circulation, these pathogenic BEVs deliver their cargo to neural circuits, heat-labile enterotoxins to dorsal root ganglia neurons, which activate TRPV1 channels to induce mechanical hyperalgesia, and miR-155 to spinal microglia, where it amplifies NF-κB signaling to sustain TNF-α and IL-1β release. This cascade weakens GABAergic inhibition and

enhances glutamatergic transmission, firmly establishing central sensitization. Furthermore, BEVs critically disrupt axonal metabolic support by impairing MCT function, leading to an energy crisis that accelerates cytoskeletal degradation and manifests as small fiber neuropathy. In a contrasting, protective role, ODEVs counteract this metabolic collapse by restoring MCT-dependent lactate transport, rescuing ATP production, and halting axonal degeneration. Simultaneously, ODEVs deliver synaptic cargo such as BDNF and miR-132 to stabilize dorsal horn neurotransmission, directly mitigating the central amplification of pain signals. Thus, in UC-associated nociplastic pain, a dynamic interplay between EVs emerges. Pathogenic BEVs drive a vicious cycle of neuroinflammation and metabolic stress that sustains central sensitization, while therapeutic ODEVs engage in the same network to restore metabolic and synaptic homeostasis (Oligodendrocyte-Derived EVs). This EV-centric dialogue across the gut-brain axis underlies the characteristic nociplastic triad in UC: stimulus-independent abdominal pain, widespread allodynia, and pain-predominant bowel dysfunction.

Beyond the gut-brain axis, EVs derived from central glial cells contribute directly to the central sensitization that defines nociplastic pain. M1-polarized MDEVs deliver NLRP3 inflammasome components to dorsal horn neurons, promoting IL-1β maturation and enhancing excitatory postsynaptic currents.[87] This action reinforces the spinal amplification that sustains pain perception long after any initiating stimulus has faded. NDEVs, for their part, transfer miR-132 to spinal neurons, relieving Ephexin5-mediated suppression of GluN2B-containing NMDA receptors and strengthening the synaptic architecture that encodes persistent pain states.[78] ADEVs from activated astrocytes carry miR-34a, which suppresses neurotrophic signaling and contributes to the dendritic instability seen in chronic central sensitization.[94] What makes these mechanisms particularly relevant to nociplastic pain is their potential to operate autonomously. Once glial activation and synaptic remodeling are established, they can persist without ongoing peripheral nociceptive input. The EV-mediated crosstalk among microglia, astrocytes, and neurons creates a reverberating circuit capable of maintaining heightened pain sensitivity indefinitely. This property distinguishes nociplastic pain mechanistically from conditions where central sensitization is more tightly coupled to peripheral drive.

A few implications follow from this EV-centered view of nociplastic pain. Targeting a single EV population is probably not enough. Suppressing pathogenic BEVs may need to go hand in hand with efforts to boost protective ODEVs or to shift MDEVs from an M1 to an M2 phenotype. The gut-brain axis is clearly a major route in this form of pain, so interventions that restore microbial balance or block BEV trafficking across the gut barrier hold considerable appeal. More generally, the involvement of multiple EV types across different compartments suggests that a narrow focus on one vesicle population misses the larger picture. What seems required is an understanding of how these signals work together as a network.

Important gaps remain. The contribution of different EV sources to specific nociplastic conditions has not been compared in any systematic way. What drives glial EVs toward a pathogenic profile when no obvious injury is present is still unclear. It is also unknown whether EV signatures in blood or cerebrospinal fluid could help separate nociplastic pain from nociceptive or neuropathic pain and inform treatment decisions. Progress will depend on better experimental models that capture the self-sustaining central sensitization seen in nociplastic states, along with clinical studies that link EV cargo profiles to careful pain phenotyping.

In short, nociplastic pain stems from altered central processing that can become self-perpetuating, sustained by maladaptive plasticity and neuroimmune dysregulation. EVs are involved at every level. BEVs translate gut dysbiosis into central sensitization through vagal and immune pathways. MDEVs and NDEVs reinforce spinal amplification. ADEVs weaken synaptic stability, while ODEVs counteract metabolic and structural decline. This multi-source framework places EVs at the center of nociplastic pain pathophysiology and points to their potential as both therapeutic targets and delivery tools for restoring normal central pain processing.

In summary, the actions of EVs in pain transcend the above pain classification: nociceptive, neuropathic, nociplastic by operating through a limited set of core pathomechanistic modules, notably neuroimmune crosstalk amplification, mitochondrial dysfunction/metabolic stress, and maladaptive neural plasticity. This perspective not only provides a more unified framework for understanding EV functions but also suggests a spotential therapeutic strategy. Targeting these shared core mechanisms, for instance by using EV-delivered inhibitors against pivotal pathways like NLRP3 inflammasome activation, could yield broad efficacy. This conceptual shift from a diagnosis-centric to a mechanism-module-

centric understanding may sets the stage for a systematic dissection of the specific molecular mechanisms through which EVs exert their analgesic effects, which will be detailed in the following Molecular Mechanisms of EV-Based Analgesia Therapy.

Molecular Mechanisms of EV-Based Analgesia Therapy

The pathogenesis of chronic pain progresses through distinct, interconnected stages. It begins with peripheral sensitization following initial tissue injury. The ensuing inflammatory cascade releases a spectrum of mediators, including bradykinin, prostaglandins, NGF, ATP, and pro-inflammatory cytokines such as TNF-α and IL-1β, which act in concert to hypersensitize nociceptors. This sensitization operates through dual mechanisms, rapid post-translational modifications like phosphorylation of ion channels like TRPV1, Nav1.8, and ASICs that immediately lower activation thresholds, and longer-term transcriptional changes that persistently enhance neuronal excitability. When this heightened peripheral drive is sustained, it precipitates central sensitization, a state of maladaptive plasticity within the central nervous system. This condition is characterized by increased synaptic efficacy in spinal dorsal horn neurons, loss of inhibitory control, and glial cell activation, collectively leading to the amplification and self-perpetuation of pain signaling, even in the absence of ongoing peripheral input. Building upon the functional repertoire of specific EV subtypes catalogued in Biological Characteristics of EVs: Source-Specific Functional Profiling and the pain pathogenesis framework established in EVs in the Pathogenesis of Pain: A Mechanistic Synthesis Across Pain Classifications, this section elucidates the molecular mechanisms by which EVs counteract these pathological processes. This section delves deep into how EVs achieve multi-level analgesia by normalizing neural signal transduction pathways, resolving neuroimmune dysregulation, facilitating structural neural repair, and restoring metabolic homeostasis in pain circuits. These distinct yet complementary mechanisms may position EVs as versatile nanotherapeutics (Figure 2).

Neural Signal Transduction

The neural pain signal transduction initiates when noxious stimuli activate peripheral nociceptors. Generated action potentials propagate along Aδ and C fibers through dorsal root ganglia to synapse in the spinal dorsal horn. Within dorsal horn laminae, primary afferents release glutamate and neuropeptides like SP, activating postsynaptic NMDA/ neurokinin 1 (NK1) receptors on second-order neurons.[158] These neurons decussate to the contralateral side, projecting signals via the spinothalamic tract through the brainstem and midbrain to thalamic nuclei. Thalamocortical projections ultimately relay information to the somatosensory cortex for discriminative processing and the anterior cingulate cortex for affective integration.

Distinct EV subtypes disrupt this cascade at strategic checkpoints. At peripheral terminals, MSC-derived exosomes carrying miRNA like miR-132 or miR-328 down-regulate Kv channels,[159] restoring nociceptor firing thresholds while promoting tissue repair as discussed in Stem Cell-Derived EVs. Along Aδ/C Fibers, nervous system-derived EVs provide autocrine support by carrying neurotrophins like BDNF to maintain axonal integrity and prevent aberrant conduction[160] as mentioned in Bacterial EVs. Within dorsal horn synapses, they shuttle Ca^{2+} channel modifiers and endocannabinoids to presynaptic terminals. This fine-tunes neurotransmitter release. For spinothalamic tract relay neurons, EVs like iMSC-EVs enhance GABAergic inhibition to suppress hyperexcitability. Although the periaqueductal gray (PAG) contains endogenous enkephalin-producing neurons that mediate descending inhibition, direct evidence that EVs deliver opioid peptides to this region remains to be established.

Hence, EVs may strategically disrupt neural pain transduction, from normalizing peripheral thresholds and fine-tuning synaptic release to enhancing spinal inhibition and potentiating descending opioid pathways. They may achieve holistic intervention across the nociceptive pathway without compromising physiological signaling.

Neuroimmune Axis Regulation

Mounting evidence has illustrated that neuroimmune interactions regulate immune and inflammatory responses. The neuroimmune axis serves as a critical interface in pain pathogenesis.[161] Immune cells dynamically interact with sensory neurons to amplify and sustain nociceptive signaling. This immune-to-neural signal initiates when tissue injury activates

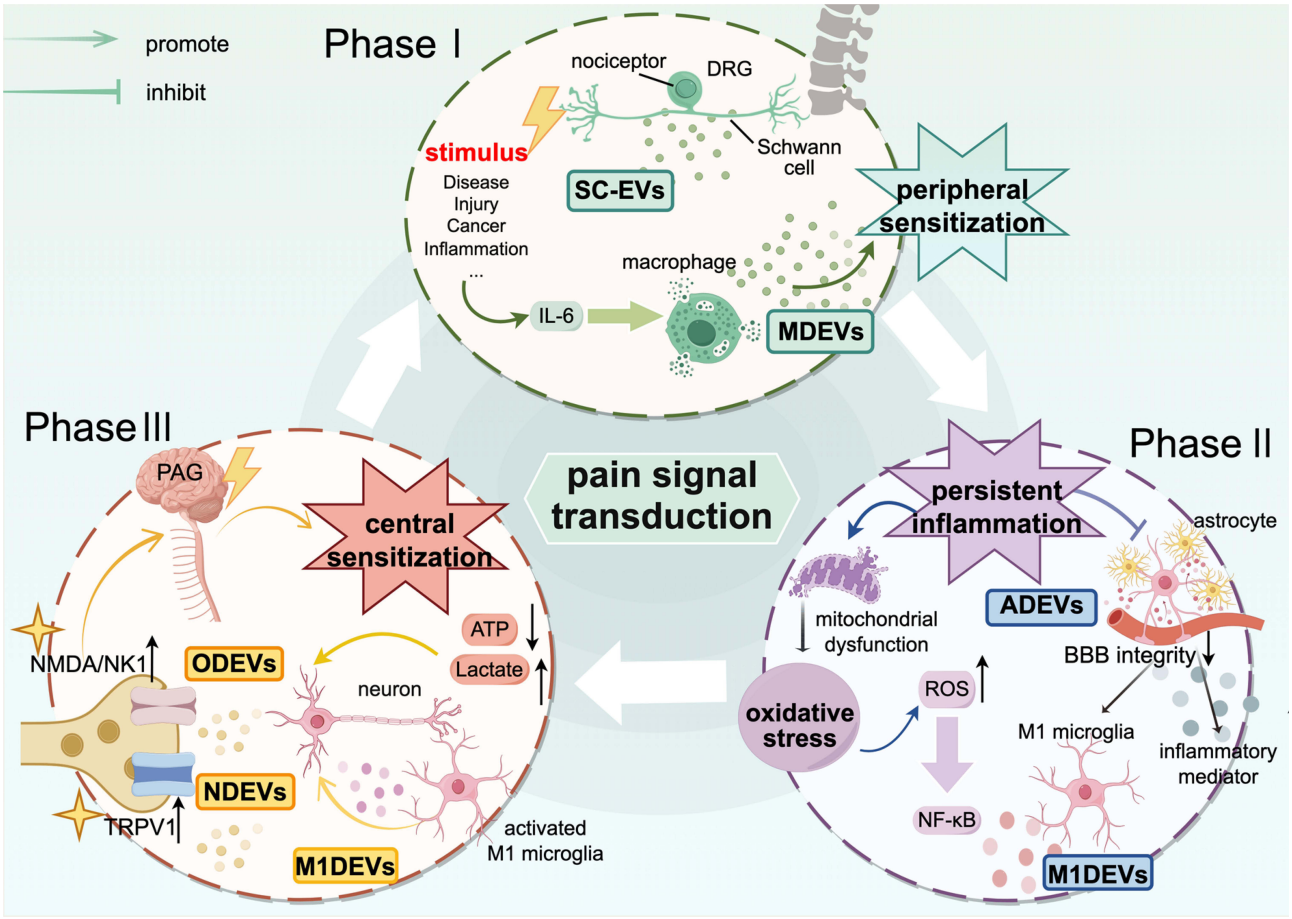

Figure 2 Different kinds of EVs in the pain signal transduction.
Abbreviations: PAG, periaqueductal gray; ATP, adenosine triphosphate; TRPV1, transient receptor potential vanilloid 1; NMDA, phosphorylated N-Methyl-D-aspartic acid; NF-κB, factor-kappa B; ROS, reactive oxygen species; BBB, blood-brain barrier; M1DEVs, microglia 1-derived extracellular vesicles; IL-6, interleukin-6; NDEVs, neuron-derived extracellular vesicles; ODEVs, oligodendrocyte-derived extracellular vesicles; ADEVs, astrocyte-derived extracellular vesicles; SC-EVs, schwann cell-derived extracellular vesicles; MDEVs, microglia-derived extracellular vesicles.

resident immune cells like macrophages and mast cells. Then, tissue injury releases inflammatory mediators like bradykinin, prostaglandins. Subsequently, these mediators immunologically sensitize sensory neurons by phosphoryling ion channels like TRPV1, Nav1.8. They lower activation thresholds and then promote neuronal hyperexcitability.[162] This establishes a feedforward loop of inflammation where inflammation lowers pain thresholds, and nociceptive signaling further intensifies inflammation. Conversely, neural-to-immune signaling occurs when neurotransmitters like catecholamines and neuropeptides bind receptors on immune cells. This further enhances pro-inflammatory cytokine production. Persistent inflammation activates innate immune cells of the CNS like microglia and T lymphocytes. Activated spinal microglia and astrocytes further augment this cycle by releasing ATP and BDNF that hypersensitize dorsal horn neurons. Microglia also release large amounts of additional pro-inflammatory cytokines like IL-1β, TNF-α, amplifying neuronal responsiveness.

EVs intrinsically regulate this neuroimmune cascade through bidirectional communication. For immune-to-neural pathways, some kinds of EVs like BEVs, MDEVs transport inflammatory cargo like miR-155, cytokines to sensory neurons as discussed in Biological Characteristics of EVs: Source-Specific Functional Profiling. This silences potassium channels and prolongs depolarization. Conversely, other kinds of EVs like NDEVs deliver regulatory miRNAs like miR-124, miR-146a to macrophages and microglia. They then reprogram them toward anti-inflammatory phenotypes. Within the spinal cord, MDEVs further modulate synaptic transmission by shuttling ATP and BDNF to dorsal horn neurons. Simultaneously, EVs like ADEVs transfer occludin/ZO-1 mRNA to endothelial cells tightening the BBB against

leukocyte infiltration. For neural-to-immune pathways, microglial EVs shuttle regulatory miRNAs to neurons, restoring potassium channel expression and normalizing excitability.[163] IMSC-EVs deliver cargo like miR-132 and NPY to microglia and T-cells. It then suppresses NLRP3 inflammasome assembly and attenuates pro-inflammatory cytokine release.

Additionally, EVs resolve maladaptive neuroimmune activation by disrupting key inflammatory hubs. They degrade NLRP3 inflammasomes through chaperone-mediated autophagy. Besides, they intercept cytokine-receptor binding via decoy ligands and restore inhibitory GABAergic tone by facilitating KCC2 membrane insertion. This coordinated intervention prevents peripheral sensitization from evolving into central hyperexcitability. Consequently, this effectively uncouple inflammation from neural hyperresponsiveness.

Therefore, EVs play a significant part in neuroimmune axis regulation. They can reestablish neuroimmune homeostasis by simultaneously quenching inflammatory drivers and reinforcing endogenous analgesic pathways.

Neural Structural Repair and Neuroprotection

Chronic pain is sustained not only by aberrant signaling but also by progressive structural compromise within neural circuits. EVs counter this degeneration through multi-level reparative mechanisms that target subcellular compartments, axons, myelin, and synapses. Specifically, they function as endogenous repair tools, transferring specific cargo like miR-219 for remyelination, NT-3 for axonal growth that actively rebuild neural architecture and restore homeostatic function.

EVs confer neuroprotection by rescuing compromised neural components through targeted molecular delivery. At the subcellular level, they deliver mitochondrial components like respiratory chain proteins and antioxidant enzymes like SOD, catalase to stressed neurons.[149] This process rescues bioenergetic deficits and reduces oxidative stress–driven apoptosis. Concurrently, EVs promote axon regeneration by transferring neurotrophic factors like BDNF, NT-3 and guidance molecules like Netrin-1, Semaphorin 3A. They activate PI3K/Akt/mTOR pathways while suppressing inhibitory RhoA/ROCK signaling. These signals and pathways enable growth cone advancement.

Furthermore, EVs facilitate repair and remyelination.[7] For remyelination, they transport cholesterol, proteolipid proteins, and miR-219 to oligodendrocyte precursors. This accelerates myelin sheath reconstitution around damaged axons to restore saltatory conduction and reduce ectopic firing. Synaptic rewiring is achieved through EV-mediated GABAergic restoration. EVs deliver KCC2 to postsynaptic neurons, reversing chloride gradient collapse and reinstating GABA-mediated inhibition.[8] Simultaneously, presynaptic vesicle trafficking is normalized via Rab GTPase delivery, stabilizing neurotransmitter release dynamics.

In a nutshell, by reconstructing structural integrity from axons to synapses and rebalancing excitation-inhibition ratios, EVs dismantle maladaptive pain circuits while reestablishing physiological neural connectivity. They serve to halt neurodegeneration and actively restore neural networks. This dual action disrupts the self-perpetuating cycle of chronic pain at its structural foundation.

Metabolic Regulation in Pain Circuits

Metabolic dysregulation within pain circuits fundamentally drives chronic pain through mitochondrial dysfunction and oxidative stress. Pathological pain states disrupt mitochondrial function in nociceptive neurons. This leads to ATP depletion and lactate accumulation, depleting cellular energy. This energy crisis impairs Na^+/K^+-ATPase activity, causing sustained membrane depolarization and spontaneous firing. Concurrently, inflammation-induced oxidative stress generates excess ROS that damage neuronal membranes, proteins, and DNA. ROS activates pro-inflammatory MAPK/NF-κB pathways and triggers mitochondrial cytochrome c release, initiating apoptotic cascades. Besides, excessive ROS can compromise BBB. This enables inflammatory cells, immunoglobulins, and complement in the blood to enter the CNS, further exacerbating neuroinflammation and damage. Simultaneously, disrupted glucose metabolism in glial cells reduces glutathione synthesis, permitting ROS to sensitize TRP channels and amplify nociceptive signals.[164]

EVs recalibrate this metabolic dysfunction through coordinated molecular interventions. For substrate restoration, they deliver glycolytic enzymes like hexokinase-2 and MCT1 to neurons, restoring ATP production while clearing lactate buildup. Besides, SEVs enhance mitochondrial energy metabolism by stimulating PGC-1α expression. This also reverse ATP depletion.[165] Mitochondrial integrity is rescued via ADSC-EVs-shuttled respiratory chain components like complex

I/IV subunits and mitophagy regulators like PINK1. They rebuild oxidative phosphorylation capacity and reduce ROS generation. Concurrently, EVs rebalance redox homeostasis by shuttling antioxidant enzymes like SOD, catalase and NADPH oxidase inhibitors that quench ROS and block MAPK/NF-κB activation.[25] Ultimately, this quenches oxidative stress and prevents excitotoxic spillover. EVs like ADSC-EVs also reestablish neurovascular coupling by transporting angiogenic factors like VEGF to endothelial cells, enhancing nutrient-oxygen delivery to hypoxic pain circuits. They concurrently modulate purinergic signaling through CD39/CD73 ectoenzymes. They convert pro-nociceptive ATP into anti-nociceptive adenosine.[166]

Hence, the metabolic rewiring by EVs spans bioenergetic restoration, redox balance, and vascular support. They collectively normalize neuronal excitability thresholds and disrupt the vicious cycle where metabolic impairment begets neural hyperexcitability. By resolving the energetic basis of pathological signaling, EVs prevent metabolic exhaustion from cementing chronic pain states.

In summary, EVs exert analgesic effects through multiple mechanisms including neural signal transduction regulation, neural-immune axis modulation, neural structural repair, and metabolic reprogramming. EVs from different sources possess unique biological characteristics, such as stem cell-derived EVs " regenerative potential, neuron-derived EVs" targeting properties, plant-derived EVs' high biocompatibility, and bacterial-derived EVs' immunomodulatory capabilities. These characteristics, detailed throughout Biological Characteristics of EVs: Source-Specific Functional Profiling–4, not only determine their roles in pain pathogenesis but also provide theoretical foundations and design concepts for subsequent engineering modifications. For instance, miR-21-5p enriched in ADSC-EVs can be utilized to design gene therapies targeting spinal cord injuries; the natural neurotactic properties of MDEVs enable anti-inflammatory siRNA delivery, while the biocompatibility of PDEVs makes them suitable for developing oral analgesic formulations. Therefore, understanding the natural mechanisms of EVs serves as both a prerequisite and bridge for designing "intelligent EEVs" to achieve precise pain management.

Potential Applications of Engineering EVs in Pain Treatment

Natural EVs boast promising therapeutic effects in modulating pain as well as efficient drug delivery capability.[167] Nevertheless, their clinical translation for pain treatment has been constrained by limited targeting ability, variable efficacy, and inconsistent biodistribution. To overcome these barriers, recent advances have focused on EEVs for targeted delivery and enhanced bioactivity in neural and immune pathways.[168] Functional optimization leverages two key approaches, surface modification and functional molecular encapsulation. Surface modification refers to attaching designed functional ligands to direct EVs to specific pain-signaling cells and activate immunity. Functional molecular encapsulation means loading therapeutic cargo like nucleic acids, protein to EVs, converting them into functional carriers for therapeutic delivery. This section will mainly explore engineering EV applications developed through these strategies to treat chronic pain states (Table 2).

Surface Modification

In a bid to enhance the targeting ability of EVs and direct EVs to specific cells involved in pain processing, researchers typically express the target protein in fusion with endogenous membrane proteins to modify the target protein on the outer surface of the EVs' membrane. Surface modification methods for EVs are generally be classified into pre-extraction modification and post-extraction modification. Pre-extraction modification refers to editing the protein expression of cells through genetic engineering methods. This typically induces high expression of targeting membrane proteins to allow the secreted EVs to express high levels of target membrane proteins indirectly.[171] For instance, the CMV-MCS-PGK-Puro lentivirus packaging system is utilized to introduce the CXCR4 gene into NIH-3T3 cells to obtain CXCR4+ EVs. This process makes them more marrow targeted. In contrast, post-extraction modification directly functionalizes isolated EVs via techniques such as lipid insertion, covalent crosslinking, or click chemistry, allowing for the precise conjugation of targeting ligands like peptides, antibodies onto the EV surface.

Surface modification of EVs represents a pivotal engineering strategy to overcome biological barriers and achieve targeted therapy in complex pain pathologies. By engineering EV membranes with tissue-specific ligands, researchers enhance their ability to overcome biological barriers and precisely target specific cell types. They have exhibit immense

Table 2 Application of Engineering Extracellular Vesicles in Pain

Modification Strategy	Disease	Tool/Method	Source	Cargo	Target	Function	Reference
Surface modification	SCI	Ang2 peptide labeled with EGFP was transfected into M0 microglia	Microglia-derived extracellular vesicles (MDEVs)	miRNA	Target spinal cord injury sites, particularly in microglia/ macrophages	Promote microglia/ macrophages phagocytosis and axon elongation and BBB repair	[28]
				Anti-inflammatory cargo	SCI sites	Alleviate secondary inflammatory responses	[28]
	DBP	Optogenetically engineered EVs	Surface-modified EVs loaded with the FOXF1 transcription factor	FOXF1	Inflamed intervertebral disc cells	Reverse mechanical hyperalgesia while restoring disc hydration and proteoglycan content	[169]
Functional molecule encapsulation	SCI	Encapsulated CCL2-siRNA within iNSCs-EVs chemically modified with the CAQK peptide	iNSCs-EVs	siRNA	SCI lesions	Protect siRNA from degradation while facilitating entry into spinal tissue	[170]
				Anti-apoptotic protein Bcl-2		Promote axonal regeneration and remyelination necessary for locomotion	
	OA	Using electroporation, siRNA was loaded into suspension-cultured 293F cell-derived EVs	Suspension-cultured 293F cell-derived EVs	MMP13-siRNA	Joint	Reduce extracellular matrix breakdown by delivering encapsulated MMP13-siRNA to chondrocytes with minimal off-target effects	[171]
	DPN	Exosomes derived from bone marrow mesenchymal stromal cells (BMSCs) were fused with PpyNps	Bone marrow-derived MSCs (BMSCs)	VEGF and NGF	Damaged nerves affected with DPN	Contribute to nerve regeneration	[172]
	Skin graft	Exosomes were isolated from the supernatant of MSCs pretreated with LPS	Bone marrow-derived MSCs (BMSCs)	Molecular markers F4/80, iNOS and CD206	Skin graft	Attenuate inflammatory infiltration, thus improving the survival of allogeneic skin graft; effectively trigger macrophage polarization into the regenerative M2 phenotype	[168]

Abbreviations: DPN, diabetic peripheral neuropathy; OA, osteoarthritis; SCI, spinal cord injury; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; DBP, discogenic back pain; BBB, blood-brain barrier; LPS, lipopolysaccharide; iNSCs-EVs, induced neural stem cells-derived EVs.

edge in ligand-receptor targeting. For instance, M2 microglia-derived EVs were engineered with Ang2 peptides to target LRP-1, a receptor markedly upregulated on activated microglia and macrophages at the lesion site after SCI. The Ang2 modification efficiency was quantitatively assessed using flow cytometry and immunofluorescence, while EV integrity and peptide display were confirmed by Western blot and transmission electron microscopy. This modification

significantly enhanced EV accumulation at the injury, as validated by in vivo imaging with a distinct peak at 6 hours post-injection.[28]

Similarly, in OA, the dense cartilage extracellular matrix has historically posed a significant barrier to efficient drug delivery to chondrocytes, which are the key cells responsible for cartilage degradation via MMP13 overexpression. To overcome this, researchers developed an engineering EV system by conjugating cartilage-affinity peptides (CAP) onto the surface of 293F cell-derived exosomes via lipid insertion, creating CAP-Exo with an encapsulation efficiency of 34.96%, about 4113 siRNA molecules per exosome.[172] Using optimized electroporation, MMP13-targeting siRNA was loaded into these EVs with high encapsulation efficiency, enabling robust nucleic acid protection and intracellular delivery. The CAP modification conferred deep cartilage penetration capacity, as evidenced by fluorescence imaging in ex vivo cartilage explants, and facilitated specific binding to chondrocytes in both monolayer and 3D culture models. In a surgically induced rat OA model, intra-articular administration of the resulting "CAP-Exo/siMMP13" system led to effective siRNA delivery into chondrocytes, significantly suppressing MMP13 expression and reducing extracellular matrix degradation. This was accompanied by enhanced synthesis of collagen type II and aggrecan, improved histological scores, and attenuation of OA-associated pain. Importantly, no notable immune reactions or systemic toxicity were observed, supporting the biocompatibility and translational potential of this targeted siRNA delivery platform. What's more, researchers fused BMSC-EVs with liposome-containing polypyrrole nanoparticles (PpyNps) to develop a novel engineering exosome system for the treatment of DPN.[169] Electrical stimulation enhances nerve regeneration after DPN by increasing the expression of growth factors such as VEGF and NGF, thereby improving angiogenesis and reducing inflammatory factors.

Beyond improved targeting, these sort of EEVs also boasts significant neuroprotective and reparative effects. Single-nucleus RNA sequencing revealed that M-Ang2-EVs promoted the expansion of oligodendrocyte precursor cells and supported neuronal differentiation. Functionally, M-Ang2-EVs enhanced myelin debris clearance by boosting phagocytic activity in microglia and macrophages, facilitated axonal regeneration, and strengthened blood–spinal cord barrier integrity via the PI3K-Akt pathway, leading to elevated expression of tight junction proteins. These mechanisms collectively contributed to functional recovery, as evidenced by improved motor and electrophysiological outcomes, alongside a shift in microglial polarization toward a protective M2 phenotype. Importantly, systemic toxicity was not observed, underscoring the therapeutic potential of this engineering EV system.[28] Similarly, intervertebral disc (IVD) degeneration is often associated with chronic low back pain, termed as discogenic back pain (DBP).[173] Surface-modified EVs loaded with the FOXF1 transcription factor achieve gender-specific pain relief. The engineering surface enables efficient, non-viral delivery of FOXF1 directly to inflamed IVD cells. This targeted delivery reverses mechanical hyperalgesia while restoring disc hydration and proteoglycan content. The functional recovery is correlated with reduced pain-related behaviors in vivo.[174] This is the first study to successfully restore tissue function while modulating pain behaviors in DBP. Such a strategy may be readily translated to other painful musculoskeletal disorders.

In a nutshell, this surface engineering works through synergistic mechanisms. Based on the mechanistic insights into EV roles across pain classifications discussed earlier, surface engineering strategies demonstrate remarkable precision in addressing distinct pain pathways. In nociceptive pain contexts, ligand-directed targeting enables concentrated EV accumulation at injury sites where inflammatory mediators activate nociceptors, effectively overcoming biological barriers that limit conventional drug delivery. For neuropathic pain conditions characterized by axonal degeneration and demyelination, surface-modified EVs not only enhance myelin clearance and promote remyelination but also restore blood-nerve barrier integrity through specific receptor-mediated interactions. Particularly in nociplastic pain states involving central sensitization and neuro-immune dysregulation, engineering EVs simultaneously modulate microglial polarization and dampen neuroinflammatory signaling while promoting neural repair mechanisms. This multi-modal approach allows surface-functionalized EVs to serve as precision nanotherapeutics that concurrently target the structural neural damage, maladaptive signaling pathways, and immune activation underlying various pain states. This may bridge mechanistic understanding with therapeutic application across the pain spectrum.

Functional Molecular Encapsulation

EVs naturally function as biological carriers by transporting molecular cargo between cells. For therapeutic applications, selecting and encapsulating appropriate functional molecules becomes critical. This encapsulation process incorporates therapeutic agents, including proteins, small molecules, or nucleic acids like miRNA and siRNA into EVs using specialized techniques. Engineering extracellular vesicles for the encapsulation of functional molecules involves two approaches, cellular engineering approaches and physicochemical approaches.[168] Genetic modification of chassis cells allows the introduction of specified intracellular molecules into vesicles during vesicle formation, enabling the encapsulation of functional molecules.

In pain therapeutics, molecular encapsulation enables precision targeting of key pathological mechanisms. Functional molecular encapsulation revolutionizes targeted pain therapy by engineering EVs to deliver precision cargo to pathological sites. They share prominent encapsulation advantages, cargo versatility, molecular protection and functional synergy. For nociceptive pain conditions, researchers preconditioned BMSC-EVs with LPS. LPS pre-Exo loaded with miRNA-222-3p was found to precise target the NF-κB pathway and inhibit the expressions of NLRP3/caspase-1/IL-1β. This promotes inflammation reduction and effectively trigger macrophage polarization into the regenerative M2 phenotype.[170] Thus, this kind of EEVs not only prolong skin graft survival but also serve as the basis for future immunosuppressive therapy to related pain.

Similarly for SCI, encapsulation merged dual therapeutic strategies, neuroprotective and anti-inflammatory properties. Researchers encapsulated CCL2-siRNA within induced neural stem cells-derived EVs (iNSCs-EVs) chemically modified with the CAQK peptide.[175] The CAQK peptide guides encapsulated siRNA specifically to SCI lesions, where EVs release their cargo into target cells. This sort of engineered "C-EVs-siRNA" leveraged natural EV properties, inherent anti-inflammatory signaling alongside synthetic components like gene-silencing siRNA. Besides, encapsulation protects siRNA from degradation while facilitating entry into spinal tissue. Simultaneously, iNSC-EVs intrinsically promote axon regrowth and blood-spinal barrier restoration. This synergy reduced neuronal apoptosis and accelerated motor recovery in mice immensely, demonstrating how molecular encapsulation coordinates multifaceted repair and pain.[12] CAQK-EVs shift microglial polarization toward reparative M2 states. Consequently, this suppresses neuroinflammation, and reduces neuropathic pain while promoting functional recovery after SCI.

In conclusion, functional molecular encapsulation represents a sophisticated engineering strategy that directly addresses the molecular mechanisms underlying different pain classifications. In nociceptive pain driven by inflammatory mediators, encapsulated anti-inflammatory miRNAs precisely regulate NF-κB signaling and inflammasome activation, effectively reducing inflammatory mediator release. For neuropathic pain characterized by neural damage, this approach enables targeted delivery of neuroprotective factors and regenerative molecules that promote axon regeneration and myelin repair. This strategy leverages EVs' natural biological properties while augmenting their therapeutic capabilities through engineering cargo loading, creating integrated nanoplatforms that concurrently address the complex molecular networks underlying various pain states. By combining targeted delivery with precision molecular interventions, functional encapsulation transforms EVs into sophisticated systems capable of simultaneously interrupting pain signaling pathways while promoting tissue repair, representing a comprehensive approach for managing complex pain conditions across different classifications.

Comparative Analysis and Synergistic Potential of Engineering Strategies

The preceding sections detail various engineering strategies in isolation. When these strategies are placed side by side, their respective strengths and weaknesses become easier to discern, and a few studies have begun to supply the kind of quantitative benchmarks that make such comparisons meaningful.

Targeting efficiency and biodistribution are two dimensions where direct comparisons can be drawn, though the data come from different disease models and should be interpreted with appropriate caution. Click chemistry-based conjugation of RGE peptide to EV surfaces achieved a 3.2-fold improvement in targeting efficiency in a glioblastoma model, with the added advantage of MRI-guided delivery.[176] DSPE-PEG lipid insertion, another widely used approach, improved blood–brain barrier penetration but showed a less favorable distribution pattern. Despite prolonged circulatory

half-life, roughly 65% of the modified EVs still accumulated in the liver and 23% in the spleen.[177] Physical fusion methods combined with sonication have yielded a 2.3-fold enhancement in tumor targeting relative to free drug, while reducing non-specific liver accumulation by 60%.[178] In terms of therapeutic response, in a rheumatoid arthritis study, thiol-maleimide-modified EVs cleared 83% of inflammatory cell-free DNA and reduced inflammation levels by 62%.[179] These figures offer useful reference points, but they are drawn from disparate experimental systems, and head-to-head comparisons under standardized conditions are not yet available.

The choice of engineering strategy is closely tied to the pain condition being treated. For nociceptive pain such as OA, CAP-modified EVs loaded with MMP13-siRNA represent one of the more thoroughly characterized approaches.[172] This system addresses two problems at once: the dense cartilage matrix that limits drug penetration, and the MMP13-driven degradation that sustains joint damage. For neuropathic pain exemplified by SCI, Ang2-peptide modified MDEVs have shown enhanced accumulation at injury sites, concurrently supporting immunomodulation, remyelination, and axonal repair.[28] CAQK-peptide guided iNSC-EVs delivering CCL2-siRNA offer a parallel strategy that couples precise targeting of the injury microenvironment with potent anti-inflammatory and neuroprotective effects.[175] For visceral pain conditions such as IBD, the landscape is less crowded. PDEVs from ginger or ginseng stand out as naturally optimized platforms for oral delivery.[139] Their intrinsic stability, biocompatibility, and multi-pathway anti-inflammatory activity, operating through TLR4/NF-κB and Nrf2 signaling, make them attractive candidates for gut-related pain disorders. What is notable across these examples is that the most effective solutions identified to date share a common logic. They pair a delivery strategy tailored to the anatomical and biochemical barriers of the target tissue with a payload selected to counteract the dominant pathological driver of that specific pain type.

Safety considerations deserve at least as much attention as efficacy metrics. Covalent modification strategies that rely on EDC/NHS-mediated amide bond formation can leave behind residual crosslinkers that promote EV aggregation and cytotoxicity if purification is incomplete. Click chemistry approaches using copper catalysts carry the risk of residual metal toxicity; copper-free variants circumvent this concern but introduce synthetic precursors such as azides whose immunogenic potential in vivo is not fully characterized.[180] Biological modification routes raise a different set of issues. Genetically engineered EVs produced through lentiviral transduction carry the background risk of vector integration, and repeated administration of vesicles displaying exogenous proteins, streptavidin being a case in point, may provoke host anti-drug antibody responses that erode therapeutic efficacy over time.[181] These are risks introduced by the engineering process itself, and they deserve careful consideration. That said, EVs as a platform do hold a baseline advantage over synthetic lipid nanoparticles when it comes to immunogenicity. Unlike artificial carriers, which tend to accumulate in the liver and trigger measurable inflammatory reactions, EVs are naturally secreted and circulate without provoking the same degree of immune surveillance. Stem cell-derived EVs have been shown in RA models to alleviate inflammation through multiple routes.[182] These routes include modulating immune cell function, suppressing cytokine release, and restoring a measure of immune balance, without the toxicity profile that accompanies many synthetic delivery systems. Whether these favorable properties persist after extensive surface engineering, however, remains an open question. For bacterial EVs, the safety calculus is different still. OMVs from gram-negative bacteria inherently carry lipopolysaccharide and other pathogen-associated molecular patterns.[183] They confer immunostimulatory properties but also pose serious endotoxin risks, limiting their clinical applicability in native form.

Encapsulating siRNA or miRNA brings its own set of problems. Loading efficiency in EVs is generally lower than in liposomal formulations, partly because EVs already carry endogenous material from their parent cells. Physical loading methods, sonication, extrusion, freeze-thaw cycles, can compound this issue. These processes often induce EV aggregation or membrane damage, creating routes for nucleic acid cargo to leak into normal tissues. Chemical modification introduces a different trade-off: excessive ligand density on the EV surface can distort membrane conformation and mask native homing receptors, undermining the very targeting specificity the modification was meant to enhance. Loading efficiency and targeting fidelity are thus entangled; pushing on one can pull on the other. Stimulus-responsive linkers offer a partial way out of this bind. Yuan et al developed a matrix metalloproteinase-sensitive linker system that achieved 89% targeted drug release at sites of corneal injury, sharply reducing off-target exposure.[184] Whether such designs can be generalized to the inflammatory microenvironments characteristic of chronic pain states remains to be seen.

Taken together, the engineering strategies surveyed here share a common theme. No single modification technique, however precisely executed, is likely to capture the full complexity of chronic pain. The condition operates across multiple anatomical compartments and engages distinct pathological mechanisms, peripheral inflammation, central sensitization, structural degeneration, that evolve over time. A scaffolded delivery platform, one that can be tailored to the spatial, molecular, and temporal dimensions of a given pain state, represents a logical next step. Whether such platforms can be built and validated in clinically relevant models remains an open question, but the conceptual tools to begin that work, source-specific functional profiling, disease-stratified mechanistic analysis, and a growing repertoire of modular engineering strategies, are now at hand. This review has sought to bring those tools together within a single comparative framework, precisely to enable such rationally designed, multi-pronged approaches.

Clinical Translation Outlook

EEVs do overcome some conspicuous shortcomings of natural EVs, such as poor targeting and variable biodistribution. Surface modification and cargo encapsulation can, under carefully optimized laboratory conditions, convert EVs into precision tools capable of navigating defined pain pathways.[171] Yet the gap between an encouraging preclinical finding and a regulatory-grade therapeutic remains substantial, and the obstacles are not purely technical.

Standardization stands out as a persistent and underappreciated problem. There is currently no consensus on how EVs should be characterized in terms of size distribution, concentration, surface marker expression, or cargo composition. Nor are there agreed-upon benchmarks for manufacturing consistency, potency assays, or release criteria.[185] This lack of standardization makes it difficult to compare results across laboratories and to establish the batch-to-batch reproducibility required for clinical development. Realistically, the field will need to converge on a set of harmonized protocols for isolation, purification, and analytical validation before EV-based products can move confidently toward regulatory review.[186]

Manufacturing at scale under GMP conditions presents a related set of challenges. Traditional EV production has relied heavily on two-dimensional cell culture systems, which are prone to cellular senescence and declining vesicle yield with extended passaging. Three-dimensional bioreactor platforms are being explored to increase culture surface area and boost EV output, but the transition from adherent flask culture to scalable suspension systems is not trivial.[187] Serum-free media, essential for clinical compliance, alter EV yield and composition in ways that demand rigorous process controls and release assays that have yet to be standardized across the industry. An ideal GMP-grade production method would generate sterile, cargo-loaded EVs in quantities sufficient for clinical trials, with minimal batch-to-batch variability and a well-defined purity profile. No such method is currently available.

The clinical evidence base, meanwhile, while growing, remains narrow. Three Phase I trials, ExoSTING, exoIL12, and EXO-CD 24, have explored EEV platforms in human subjects. These studies provided valuable initial signals, but their intratumoral or inhalation-based delivery routes limited what could be learned about systemic immunogenicity and long-term safety.[188] More recently, a Phase I trial of ILB-202, an engineering EV designed to inhibit NF-κB signaling, adopted a double-blind, randomized, placebo-controlled design. It enrolled 18 healthy volunteers and generated encouraging early data on safety and anti-inflammatory activity.[189] As the first clinical investigation of systemically administered, allogeneic engineering EVs, this trial represents a meaningful step forward. It also underscores how early the field remains. Robust pharmacokinetic data, biodistribution profiles in target tissues, and the effects of repeated dosing in patient populations are not yet available from any published study. Large multicenter trials that could validate EV-based strategies in pain or related inflammatory conditions have not been completed.

Regulatory pathways add further uncertainty. EV-based products occupy an ambiguous space between traditional biologics and cell-based therapies. To date, no EV therapeutic has received regulatory approval for any indication. Without a critical mass of clinical data from well-powered trials, the path forward will involve navigating a regulatory landscape that is itself still taking shape.

Targeted delivery, despite substantial engineering progress, continues to face physiological barriers. Systemically administered EVs are cleared from circulation by the liver and spleen, and crossing the BBB in meaningful quantities remains a challenge.[190] LRP1-mediated clearance and other scavenging mechanisms sharply curtail EV accumulation at pathological sites.[28] Several recent developments offer grounds for measured optimism on this front. Dual-ligand

modification strategies, reported in a 2025 ACS Nano study, have shown that engaging two distinct targets on the brain endothelium can enhance BBB penetration beyond what single-ligand approaches achieve.[191] In parallel, allosteric targeting strategies that exploit transmembrane domain interactions have emerged as an alternative that may circumvent the problems of endogenous ligand competition and receptor shedding that limit conventional receptor-targeting methods.[192] The mechanisms through which these strategies operate are not yet fully mapped. An emerging line of work on EEV-mediated modulation of autophagy in brain endothelial cells suggests that barrier function can be influenced through pathways beyond simple ligand–receptor engagement, adding a further layer of complexity to the design problem.[62] These advances are encouraging, but they have been demonstrated almost exclusively in rodent models. Translation to human physiology will require extensive validation.

Looking further ahead, technological convergence may offer new avenues for progress. Artificial intelligence and machine learning approaches are beginning to be applied to the analysis of complex multi-omic datasets derived from EV cargo, proteomic, metabolomic, and transcriptomic profiles that could in principle reveal subtle signatures predictive of disease onset, progression, or therapeutic response. In related fields, machine learning-assisted analysis of EV surface antigens by flow cytometry has enabled reliable monitoring of acute cellular rejection following heart transplantation.[193] Whether such tools can be adapted to pain indications remains to be seen, but the potential is worth noting. The integration of AI-driven analytics with large-scale EV datasets may, over time, help identify patient populations most likely to benefit from EV-based interventions and accelerate mechanistic insights.

The strategies discussed above converge on a simple point. Chronic pain involves peripheral inflammation, central sensitization, and structural degeneration, often within the same patient. No single engineering approach addresses all three. What works for one compartment may miss another entirely. A multi-layered delivery architecture, one capable of adapting to the spatial, molecular, and temporal profile of a given pain state, represents a compelling but demanding design target. Whether such architectures can be built and validated in large animal models remains an open question. Answering it will require not only modular engineering capabilities, like source-informed EV selection, disease-specific analysis, and controlled cargo loading, but also a deeper understanding of EV biodistribution, circulatory half-life, and clearance mechanisms under physiologically relevant conditions. Long-term safety studies that systematically evaluate off-target effects, immune activation, and cumulative toxicity of bioengineered formulations are equally important and, at present, scarce. Progress will depend on interdisciplinary collaboration: microfluidic workflows that standardize EV isolation and modification, low-immunogenicity materials that reduce host recognition of engineered surfaces, and machine learning tools that accelerate the optimization of ligand-receptor interactions. Regulatory frameworks for genetically modified EV products are still in flux, and clear safety and efficacy guidelines will need to be established in parallel with technological development. The present review has brought source-specific profiling, disease-stratified analysis, and modular engineering strategies together under one comparative framework. The hope is that this synthesis provides a useful starting point for the multi-pronged designs that the complexity of chronic pain demands.

Conclusions and Further Perspectives

Beyond the broad expectation that extracellular vesicles may prove useful for pain, the evidence assembled here points to a more specific set of conclusions. A clear hierarchy of mechanistic specialization has emerged across EV sources. ADSC-EVs and BEVs show their strongest effects where metabolic dysregulation and barrier disruption dominate, diabetic neuropathy and visceral inflammatory pain, respectively. UC-MSC-EVs and IMSC-derived EVs excel in settings that require precise immunomodulation, particularly macrophage polarization and inflammasome restraint. BMSC-EVs and SC-EVs are distinguished by their capacity for structural repair, with the most consistent axonal regeneration and remyelination data originating from SCI and peripheral nerve trauma models. MDEVs occupy a unique position. Their functional output mirrors the polarization state of the parent cell, rendering them simultaneously a therapeutic opportunity and a source of pathogenic signaling that demands careful management. These source-dependent functional profiles, catalogued and compared in this review, provide a rational basis for matching EV types to specific pain etiologies rather than treating all EVs as interchangeable entities.

The three ICD-11 pain categories are not equally addressed by the current literature. Nociceptive pain, especially osteoarthritis, has attracted the most extensive preclinical investigation, and the mechanisms by which stem cell-derived

EVs modulate synovial inflammation and cartilage degradation are now comparatively well defined. Neuropathic pain has also received substantial attention, though the evidence remains concentrated in acute and subacute injury models rather than established chronic neuropathic states. Nociplastic pain represents the most conspicuous gap. Beyond the role of BEVs in gut-brain axis signaling, few studies have examined how CNS-derived or engineering EVs might intervene in the self-sustaining central sensitization that characterizes fibromyalgia, irritable bowel syndrome, and related conditions. This imbalance warrants attention, given that nociplastic pain is both clinically prevalent and mechanistically distinct. Among engineering strategies, surface modification with tissue-specific ligands has generated the most compelling targeting data in preclinical pain models, the Ang2 peptide system for SCI and the cartilage-affinity peptide platform for OA being the standout examples. Functional encapsulation strategies offer versatility but lag behind synthetic nanoparticle formulations on quantitative metrics of loading efficiency and release kinetics, and off-target effects of encapsulated nucleic acids remain insufficiently studied. No combination strategy, spatial, functional, or temporal, has yet been tested experimentally in pain models.

Across all EV sources and pain categories, a shared set of translational deficits persists. Pharmacokinetic data in large animals are sparse. Biodistribution studies in humans are absent. No Phase II trial of an engineering EV for any pain indication has been completed; the only Phase I evidence comes from a single trial of systemically administered allogeneic EVs in healthy volunteers. Long-term safety profiles, including immunogenicity after repeated dosing and cumulative off-target effects, remain uncharacterized. These gaps are not peripheral qualifications to an otherwise positive picture. Instead, they are central obstacles that the field must address before clinical translation can be responsibly discussed.

The work ahead is best understood as a set of parallel efforts. Standardized GMP-compliant manufacturing platforms that ensure batch-to-batch consistency in cargo composition and potency are foundational. Pharmacokinetic and toxicological characterization in large animal models under repeated dosing regimens is equally urgent. Clinical protocols that incorporate mechanistic biomarkers, like EV cargo signatures, inflammatory mediators, and quantitative sensory testing, will be needed to connect molecular actions to patient-relevant outcomes. Nociplastic pain deserves particular attention, both as an area of unmet clinical need and as a rigorous test of whether modulating central neuroimmune signaling translates into meaningful analgesia. This review has brought source-specific profiling, disease-stratified analysis, and modular engineering strategies together within a single comparative framework. The intent is not to declare any single approach superior, but to provide a structured basis for making deliberate, evidence-informed decisions about which tools to deploy against which dimensions of chronic pain. Subjecting those decisions to rigorous experimental and clinical scrutiny is the task that now lies before the field. As manufacturing standards mature and clinical evidence accumulates, it can be expected that EV-based strategies may begin to offer genuinely new and non-opioid options for pain conditions that have resisted conventional pharmacotherapy.

Data Sharing Statement

No datasets were generated or analyzed during the current study.

Consent for Publication

All authors have approved the manuscript be submitted.

Acknowledgments

We would like to express our gratitude to Figdraw for its assistance in generating the image (https://www.figdraw.com/static/index.html#/). The image ID is IARIIc4466; YOTAPa844b.

Funding

This work was supported by the National Natural Science Foundation of China (82305423, 82274670, 82305424, 82575242), Yangfan Special Project of Shanghai Science and Technology Innovation Action Plan (23YF1447600, 24YF2740700), Shanghai Key Laboratory of Traditional Chinese Medicine Tuina Techniques for Musculoskeletal Disorders (24dz2260200).

Disclosure

The authors declare no competing interests.

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