

REVIEW

The role of exosomes in intercellular and inter-organ communication of the peripheral nervous system

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(Received 1 November 2021, revised 9 December 2021, accepted 23 December 2021, available online 21 January 2022)

doi:10.1002/1873-3468.14274

Edited by Andreas Herrlich

Exosomes, nano-sized extracellular vesicles, are produced via the endosomal pathway and released in the extracellular space upon fusion of multivesicular bodies with the plasma membrane. Recent evidence shows that these extracellular vesicles play a key role in cell-to-cell communication. Exosomes transport bioactive proteins, mRNAs, and microRNA (miRNAs) in an active form to adjacent cells or to distant organs. In this review, we focus on the role of exosomes in peripheral nerve maintenance and repair, as well as peripheral nerve/organ crosstalk, and discuss the potential benefits of exploiting exosomes for treating PNS injuries. In addition, we will highlight the emerging role of exosomes as new important vehicles for physiological systemic crosstalk failures, which could lead to organ dysfunction during neuroinflammation or aging.

Keywords: exosome; homeostasis; intercellular communication; PNS; regeneration

The evolution of mammalian organisms has seen them develop an astonishing degree of complexity, involving hundreds of highly specialized cell types working in synergy [1–3]. On both the organism scale and at the level of individual organs or tissues, interplay between different cells is required for development, maintenance, and repair [4–7]. To this end, cells must communicate and exchange information with each other in order to coordinate a myriad of possible cellular processes [6–8].

Cell-to-cell communication involves a variety of mechanisms, ranging from the secretion of individual molecules to the release of membrane-coated vesicles [9,10]. While orderly intercellular transfer of information is fundamental to a functioning organ and organism, erroneous communication between cells might be implicated in or, in some cases, even be causative of pathological conditions and diseases [4].

In the past ten years, exosomes have been identified as key players in intercellular and long-distance communication of cells and their environment through the transfer of information [11,12]. Since their discovery in the 1970s by Rose Johnstone, there has been an exponential gain in knowledge of exosomes and their function in physiological and pathological conditions [13]. Perhaps most prominently, exosomes have been studied for their involvement in various aspects of cancer biology [14].

Compared to an average-sized cell, exosomes may appear miniscule. However, compared to other molecules and compounds that undergo secretion by cells (e.g., hormones and cytokines), their cell type-specific and diverse content, which consists of proteins, mRNA, miRNA, and DNA, emphasizes their potential involvement in a multitude of biological functions [12]. By virtue of their nano-scale size and biologically

Abbreviations

ALS, amyotrophic lateral sclerosis; DRG, dorsal root ganglia; GBS, Guillain-Barré syndrome; MSC, mesenchymal stem cell.

versatile cargoes, exosomes are capable of reprogramming and/or modulating cellular processes in recipient cells in an organism-wide manner [15,16].

Our review aims to summarize existing data on exosomes in the peripheral nervous system (PNS) and their functional significance in intercellular and inter-organ communication in health and disease.

General aspects of exosome biology

Exosomes are nano-sized vesicles that are secreted by virtually all cell types into the extracellular space, under both steady-state and pathological conditions. As such, they belong to a greater entity of extracellular vesicles which includes apoptotic bodies and microvesicles. These extracellular vesicles can be differentiated and categorized by their size, cargo, and cellular origin [17,18]. However, nomenclature in the literature is inconsistent as it is difficult to separate the different types of extracellular vesicles under experimental conditions [19,20]. The International Society for Extracellular Vesicles have published guidelines to make extracellular vesicle research comparable and reproducible proposing experimental conditions or controls [21]. In this review, we will use the term exosome even if the endosomal origin was not proven experimentally.

Exosomes derive from the endosomal pathway of cells and display a diameter of 30–100 nm [22–24]. Enclosed by a lipid bilayer membrane, they convey complex cellular signals from their parent to their recipient cells. Following secretion from their parental cells, exosomes are detectable in all bodily fluids including plasma, saliva, urine, pleural ascites, amniotic fluid, cerebrospinal fluid, colostrum, breast milk, semen, and lymphatic fluid [25]. With regard to their biological stability, a wide range of half-lives has been reported for exosomes, ranging from a few minutes [26] up to several hours [27], until they are taken up by target cells.

Exactly how exosomes exert their function mechanistically on target cells is not yet fully understood; however, an array of stimulatory and inhibitory functional outcomes have been shown to be induced by exosomes. These include cell proliferation, angiogenesis, apoptosis, cytokine production, modulation of immune reaction, preparation of a metastasis-supporting microenvironment, and even the determination of organ specificity in metastasis [28]. Exosomes are believed to act on their recipient cells not only upon internalization through fusion, receptor-mediated endocytosis, macropinocytosis, or phagocytosis but also by cleavage of surface-bound components of exosomes, or binding of exosome surface-

associated molecules to receptors on the target cell [25].

Under pathological conditions, the abundance and content of exosomes may vary extensively in comparison to healthy cells under steady-state conditions [22,24]. Perhaps most impressively, cancer cells secrete up to 1000-times more exosomes than noncancer cells [29]. Functionally, exosomes released from cancer cells have been shown to support cancer progression at multiple steps. In line with this concept, the number of circulating exosomes in the blood plasma of human colorectal cancer patients correlates with poor prognosis and shorter survival [30].

Exosome biogenesis and composition

Exosomes serve as molecular cargoes for intercellular communication and may therefore contain a wide variety of biomolecules on top of and inside their lipid bilayer membrane. Containing proteins, miRNAs, mRNA, DNA, and cytokines [31,32], over 4500 proteins, 1600 mRNAs, and 760 miRNAs have been detected in association with exosomes [33].

Exosomes originate from the endosomal pathway of cells. They emerge during the process of endocytosis and can further morph into intraluminal vesicles (ILV) via inward budding of the endosomal membrane. The resultant multivesicular bodies (MVBs) can either fuse with lysosomes, leading to degradation of their content, or merge with the plasma membrane to release exosomes [23]. Each individual step involves a complex molecular machinery and is subjected to tight regulation.

Given their cellular origin, exosomes carry some endosome-associated proteins that allow for their identification, such as the tetraspanin proteins CD63, CD9, and CD81, the heat shock proteins 70 (Hsp70), tumor susceptibility gene 101 (Tsg101), and ALIX [34,35,34,35]. However, none are exosome-specific as they are also found on other extracellular vesicles [17] and it might be impossible to completely separate the different subtypes experimentally [36].

Precisely how their content is sorted into exosomes is not fully understood. Originally, exosomes were thought to remove waste products from cells. Recent experimental work further revealed a major role of the syntenin/syndecan pathway in the formation of ILVs. Here, syntenin binds directly to ALIX, which is a link to the endosomal-sorting complex required for transport (ESCRT) machinery [37,38]. MVBs contain proteins and RNA from the cytoplasm, but other proteins from the Golgi or endoplasmic reticulum (ER) are also sorted into MVBs [25]. While endosomal sorting is usually mediated by the ESCRT machinery,

ESCRT-independent pathways have also been described, such as a ceramide/tetraspanin-dependent pathway [23,25,38]. RNAs can also be sorted into exosomes by several mechanisms, which can be mediated by KRAS-MEK and Argonaut 2 (Ago2) [39] or other RNA-binding proteins such as annexin A2 or major vault protein (MVP) that recognize specific RNA sequences or structures [40]. In addition, nonselective loading of RNA into exosomes occurs passively due to local RNA concentrations in the cytoplasm [40,41]. Intercellular trafficking of MVBs and fusion with the plasma membrane involves various protein complexes, such as Rab-related proteins (Rabs) for trafficking and Sec1 proteins and SNAREs for fusion [42,43].

The role of exosomes during peripheral nerve homeostasis

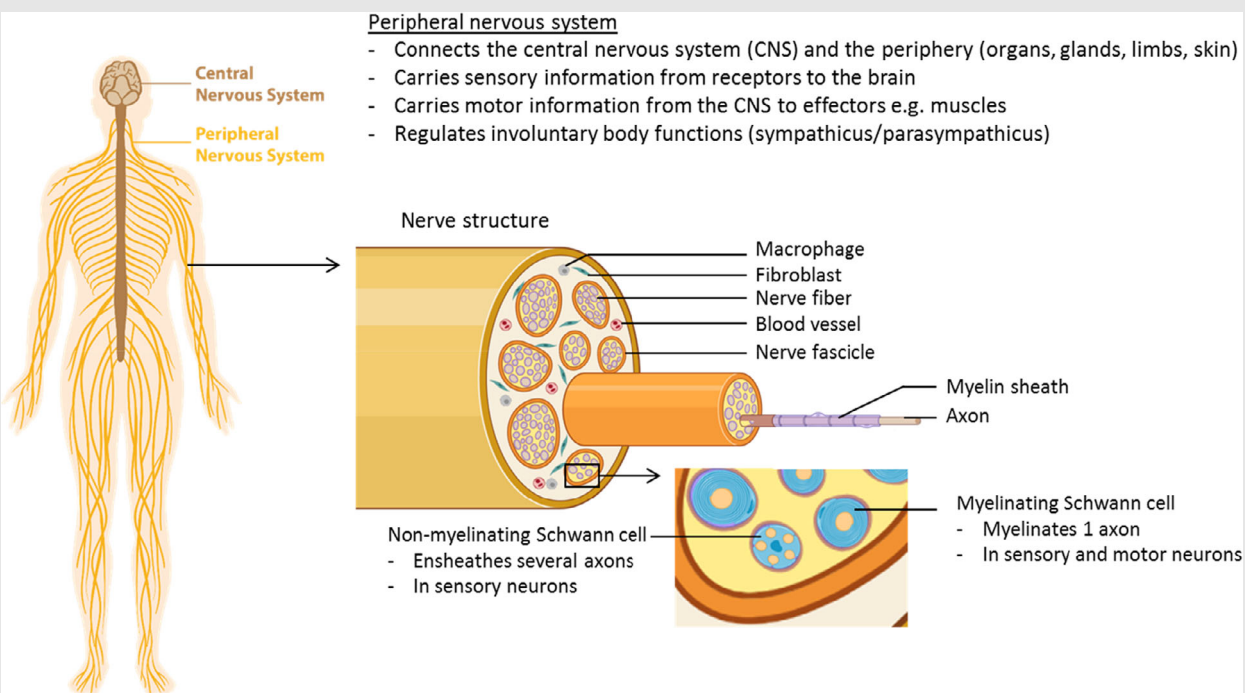
The peripheral nervous system (PNS) refers to all nerve fibers and structures outside the central nervous system (CNS), that is, the brain and the spinal cord (Box 1). On a cellular level, the former comprises a variety of different cell types working in synergy.

Peripheral nerves consist primarily of axons—specialized extensions of neuronal cells—as well as Schwann cells—the main glial cell type of the PNS [44]. Schwann cells not only ensheath the axons but also provide trophic and metabolic support [45].

As their interaction is crucial to the functional and structural integrity of nerves throughout lifetime, the communication between glial cells and neurons is essential in both physiological and pathological conditions [46,47]. Bidirectional signaling between axons and Schwann cells involves different mechanisms. Paracrine signaling for small molecules such as ATP and activation of the appropriate receptors have been reported [48], as well as physical coupling, for example, via gap junctions or adhesion molecules present at the paranodal region of myelinated axons [49]. Recent years have seen communication via exosomes come into focus. Schwann cells secrete exosomes that can be taken up by axons *in vivo* and *in vitro* [50]. Exosomes released from Schwann cells are involved in the regulation of Schwann cell migration, those from differentiated Schwann cells exhibit an altered miRNA signature within the exosomes, compared to undifferentiated

Box 1. Peripheral nervous system

The peripheral nervous system fulfills a variety of different functions. The peripheral nerve structure is summarized here including different cell types. The figure was created with BioRender.com.



Schwann cells and inhibited the migration of Schwann cells *in vitro* [51]. There is also evidence that Schwann cells provide ribosomes to the axons via exosomes, as ribosomal proteins have been detected in Schwann cell-derived exosomes [52].

In addition to these two main cell types in the peripheral nerve, resident and infiltrating immune cells, endothelial cells forming blood vessels, and fibroblasts also contribute to the structural integrity and functionality of the PNS [44]. However, studies have focused on their role during regeneration or disease, which we discuss in the following.

The role of exosomes during peripheral nerve regeneration

Peripheral nerves are key structures for transmission of signals between organs or tissues and the central nervous system (CNS) and remain relatively stable throughout life. In contrast to the CNS, they possess high regenerative capacity after injury to recover sensory and motor functions [53]. In the process of Wallerian degeneration, the axon and myelin sheath distal to the injury site become fragmented, while Schwann cells and macrophages proliferate and phagocytose the degraded material. Schwann cells then form bands of Büngner to guide the axonal sprouting and enable reconnection with the target organ, followed by remyelination of the axon [54]. Schwann cells play a major role during this regenerative process. There are two types of Schwann cells: Myelinating Schwann cells are the main cell type within the peripheral nerves (~ 50%) that myelinate large axons, whereas non-myelinating Schwann cells (~ 20%) bundle together smaller axons (Remak bundles) [55,56].

Following injury, the high plasticity of Schwann cells is integral to efficient regeneration. They can switch into a proliferative, reactive cell that fosters, for example, the guidance of axons [57] and remodeling of the nerve environment [58]. Following axonal reinnervation of the target, the dedifferentiated Schwann cells redifferentiate into myelinating Schwann cells to myelinate the newly formed axon and maintain homeostasis [59,60]. In addition to Schwann cells, all other cell types of the peripheral nerves (macrophages, fibroblasts, endothelial cells, pericytes) respond to injury with proliferation and contribute to an efficient regeneration [60,61]. In the following, we discuss how exosomes contribute to an efficient regeneration between these different cell types of the peripheral nerves.

As the major cell type within the peripheral nerves, Schwann cells are known to secrete exosomes that are important for axonal regeneration. Schwann cell-

derived exosomes have been shown to increase neurite outgrowth *in vitro* [50]. The authors confirmed these results in a crush injury model wherein Schwann cell-derived labeled exosomes enhanced axonal regrowth (2-times longer neurites) and improved nerve function, indicated by a pinch test. They further indicated specificity of the exosomal communication, since fibroblast-derived exosomes had no effect on axonal regeneration and RhoA GTPase activation (which inhibits axonal elongation [62] was only decreased in the Schwann cell-derived exosome-treated group. Furthermore, it was observed that Schwann cells formed a vesicle-like structure containing labeled ribosomes that were budding from the Schwann cell toward the axon after an injury [63]. They anticipate that these ribosomes are important for an immediate regeneration response [63] as axons depend on local translation of proteins from mRNA essential for regeneration such as many cytoskeletal proteins [64,65]. As the axonal transport of proteins is slow [66], the support of the axons with ribosomes from Schwann cells via extracellular vesicles could support the local protein synthesis after an injury [63].

After nerve injury, miRNA expression levels are changed within Schwann cells [67,68]. Several miRNAs are shown to be important to regulation of cell debris removal after injury, as well as Schwann cell proliferation or homeostasis [69,70]. Specifically, miR-340 positively regulated cell debris removal at the injury site and axon growth *in vivo* [70]. Furthermore, the miR-221/222 cluster was significantly changed at the injury site and shown to affect Schwann cell proliferation and migration *in vitro* [69]. The transition of Schwann cells firstly to a reactive and then back to a myelinating type is crucial for efficient regeneration. Here, miRNAs seem to be involved. A lack of miRNAs in Schwann cells prompts myelination deficits *in vivo* due to reduction of Krox20—a promyelination factor—and increase in myelination inhibitors such as SOX2, Notch1, and Hes1 [71,72]. However, the underlying mechanisms of such communication are not yet understood including whether miRNAs could be transported to other cells via exosomes. Nonetheless, it is reasonable to assume that exosomes and their miRNA cargo are important modulators during peripheral nerve regeneration. A recent publication demonstrated that changes in the miRNA cargo of Schwann cell-derived exosomes can influence neurite growth [73]. The authors demonstrated that exosomes derived from repair Schwann cells enhanced neurite outgrowth, but not exosomes from differentiated Schwann cells *in vitro*. They further demonstrated involvement of the miRNA cargo of the exosomes, specifically, miR-21

was strongly upregulated in exosomes derived from repair Schwann cells compared to differentiated Schwann cells. These modulate the pro-growth effect in neurites by downregulating PTEN and activation of PI3K in the neurons. Several other miRNAs have also been shown to be changed within repair Schwann cell-derived exosomes. However, their functions have not yet been investigated nor the effect on other cell types within the nerve.

Other cell types also communicate via exosomes during peripheral nerve regeneration. In the case of dorsal root ganglia (DRG) sensory neurons, exosomes have been demonstrated to contribute to communication between sensory neurons and macrophages after damage to the peripheral nerve by microRNA upregulation [74]. Here, the authors found that macrophages phagocytose sensory neuron-derived exosomes and the increase in miR-21-5p expression supports a shift toward a pro-inflammatory phenotype. These pro-inflammatory macrophages are especially important for clearing cellular debris after a nerve injury and provide a suitable microenvironment for tissue repair [75]. Furthermore, it has been shown that macrophages release exosomes that mediate ROS signaling during nerve regeneration [76]. The authors showed that after nerve injury macrophages secrete exosomes containing active NADPH oxidase 2 (NOX2) complexes which can be taken up by DRGs via endocytosis and were required for the neurite outgrowth. They propose a model where NOX2 inactivates PTEN via oxidation and therefore stimulation of PI3K-Akt signaling to promote axonal regeneration.

Taken together, exosomes are important modulators during peripheral nerve repair, which allow communication between different cell types and provide a suitable microenvironment for regeneration.

Exosomes in inter-organ crosstalk from and to peripheral nerves and their potential therapeutic benefits

Exosomes not only modulate homeostasis and response to injury at the site of injury but can also be transported systemically throughout the body.

Stem cells of different types represent an interesting source of exosomes that can promote neuronal growth or survival in several studies, which would be beneficial for nerve repair or homeostasis. Mesenchymal stem cells (MSCs) from bone marrow and sources such as umbilical cord, menstrual stem cells, and chorion stem cells have been shown to secrete exosomes that promote neurite outgrowth in primary neuronal cultures (DRG or cortical culture) [77]. Reminiscent of

the CNS, adipose-derived MSC exosomes have been shown to increase neurite outgrowth and sciatic nerve regeneration after injury [78]. This research showed that the exosomes were taken up by Schwann cells and enhanced their proliferation *in vitro*. *In vivo*, exosomes were internalized by axons and were beneficial for axonal regeneration and functionality. Bucan *et al.* further demonstrated that the adipose-derived MSC exosomes carry neurotrophic factors that seem to contribute to the improved peripheral nerve regeneration. Similarly, gingiva-derived MSC exosomes promoted axonal recovery in a sciatic nerve crush injury model [79]. Here, exosomes promoted proliferation and migration of Schwann cells by upregulation of characteristic genes of repair Schwann cells such as c-Jun, Notch1, Sox2, and GFAP.

MSC-derived exosomes are also shown to play a role during neuroinflammation [80]. These exosomes were involved in the regulation of macrophage plasticity [81] and polarization of microglia (immune cells in the CNS) toward an anti-inflammatory phenotype [82] to mediate neuroinflammation.

It is also possible to use MSCs to differentiate them into a Schwann cell-like phenotype [83]. Their exosomes promoted neurite outgrowth *in vitro*. The use of differentiated MSCs instead of Schwann cells means avoiding the sacrifice of healthy nerve tissue for potential therapy.

Although most studies investigated the CNS, it is reasonable to assume that the use of MSC exosomes could also be useful for treatment of peripheral nerve injuries. However, whether MSC-derived exosomes also contribute to nerve homeostasis under physiological conditions remains unknown.

Lessons from the CNS—Speculations about exosome-mediated PNS inter-organ crosstalk, regeneration, disease involvement, and aging

There is very little known about the communication from and to the peripheral nerves via exosomes. Therefore, we will try to link research from the CNS and speculate about potential exosomal functions in the PNS.

With regard to motor function, the transmission of information from synaptic endplates of motor neurons to the neuromuscular junction (NMJ) of muscles, exosomes can transfer membrane proteins from neurons to NMJ in *Drosophila* [84]. Furthermore, circulating immune cells release exosomes that are beneficial for CNS myelination *in vitro* [85]. Evidence also exists that Schwann cells communicate with the CNS

via exosomes [86]. Specifically, proteomic analysis of Schwann cell-derived exosomes revealed the presence of several proteins associated with CNS repair, including axon regeneration and inhibition of inflammation. These findings could be useful in developing novel therapeutic approaches for CNS and PNS injuries.

Exosomes also contribute to angiogenesis during regeneration. Indeed, several studies indicate that exosomes and their miRNA cargo improve angiogenesis and neurogenesis in the brain [87,88]. Mesenchymal stem cell-derived exosomes promoted angiogenesis in the brain [87], and neurons have been shown to secrete exosomes containing miR-132 to endothelial cells, which is important for vascular integrity [88]. Whether exosomes also promote angiogenesis in the peripheral nerve remains to be determined.

While exosomes are found to be communication tools between different organ systems, little is known of their physiological role, particularly within the peripheral nervous system, as most studies address pathological conditions.

Disease involvement and aging

The functional significance of exosomes for proper communication of PNS cells with other cells outside or within the PNS is highlighted by mounting evidence of altered exosome biology in various diseases.

In Guillain-Barré syndrome (GBS), the most common and severe acute paralytic neuropathy, immune-mediated processes lead to the damage of peripheral nerves. In an animal model for GBS in rats, experimental autoimmune neuritis (EAN), exosomes released by M1-type macrophages have been shown to aggravate disease pathology by enhancing Th1 and Th17 response, in comparison to M2-type macrophage-derived exosomes [89].

In amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease, the degeneration of the upper (CNS) and lower (PNS) motor neurons causes progressive paralysis [90]. Beyond the exclusive damage of neuronal cells, a more complex pathomechanism has emerged. For example, prion-like propagation of misfolded proteins between cells has been linked to the development or spread of ALS or other neurodegenerative diseases [91]. In roughly 20% of inherited cases SOD1 mutations contribute to the development of ALS. Here, it has been shown that the misfolded protein can also be spread by exosomes [92]. Furthermore, ALS muscle cells exhibit changes in exosome quality and quantity and the exosomes have been shown to be toxic to motor neurons, [93]. These findings suggest

that exosomes from the skeletal muscle contribute to the spread of ALS and neuronal toxicity.

During aging, the risk of developing neurodegenerative diseases increases. There is evidence that exosomes are involved in neurodegenerative processes; they are important vehicles in cell-to-cell communication and can influence the gene expression patterns of their target cells [94] and contribute to inflamm-aging [95]. However, our understanding of the physiological and pathological roles of exosomes is in its infancy. Whether exosomes are neurodegenerative or neuroprotective is not yet understood and debated in the literature. On the one hand, it has been shown that exosomes can carry disease-associated factors, toxic molecules, or pro-inflammatory cytokines that are implicated in aging or neurodegenerative diseases [96,97]. The disease could be triggered by a dysregulation in cell-to-cell communication between neurons and glia cells via exosomes, for example. On the other hand, exosomes can also distribute neuroprotective factors to their target cells [98]. Certainly, exosomes are important mediators in cellular communication during aging and disease development. However, understanding the mechanisms of exosome regulation and their systemic effects will be part of future studies.

Conclusions and perspectives

Exosomes have become the focus of much research in recent times, with increasing numbers of publications on the role of exosomes during pathogenesis and a burgeoning interest in the identification of exosome biomarkers. This would help clinicians in making diagnoses, patient stratification, decisions on treatment options, or in monitoring regenerative processes for PNS and other pathologies. Furthermore, understanding the mechanisms of exosome release and targeted uptake might enable modulation of exosome release in the future to treat diseases. Furthermore, cell-free therapy with MSC-derived exosomes that exhibit a regenerative potential in several tissues might be possible [99]. In the meantime, it remains necessary to achieve a better understanding of the local and systemic roles of cell type-specific exosomes, which in turn would afford an understanding of which cell types communicate with each other and under which conditions.

Currently, biomarker identification is based on a mixture of exosomes from different cells (e.g., the blood or cell culture experiments) that might not completely cover the *in vivo* situation. Therefore, it would be necessary to label exosomes cell type-specifically so as to monitor them under physiological and pathological conditions and compare their cargoes. Robust

standardized isolation methods for the isolation of small amounts of exosome, making identification of biomarkers more comparable, would be a welcome advantage. Understanding the role of exosomes as important players in cellular communication under physiological and pathological conditions may help to develop therapies for tissue regeneration and age-associated diseases.

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