

# Exosomes as Neurological Nanosized Machines

Elham Pishavar, Martina Trentini, Federica Zanotti, Francesca Camponogara, Elena Tiengo, Ilaria Zanolla, Massimo Bonora, and Barbara Zavan\*



Cite This: *ACS Nanosci. Au* 2022, 2, 284–296



Read Online

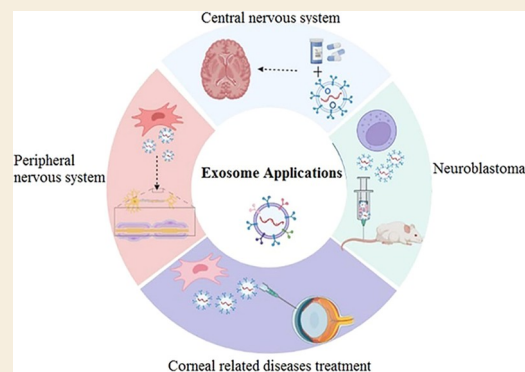
ACCESS |

Metrics & More

Article Recommendations

**ABSTRACT:** In the past few decades, nanomedicine research has advanced dramatically. In spite of this, traditional nanomedicine faces major obstacles, such as blood–brain barriers, low concentrations at target sites, and rapid removal from the body. Exosomes as natural extracellular vesicles contain special bioactive molecules for cell-to-cell communications and nervous tissue function, which could overcome the challenges of nanoparticles. Most recently, microRNAs, long noncoding RNA, and circulating RNA of exosomes have been appealing because of their critical effect on the molecular pathway of target cells. In this review, we have summarized the important role of exosomes of noncoding RNAs in the occurrence of brain diseases.

**KEYWORDS:** exosomes, mesenchymal stem cells, neurological, brain diseases, extracellular vesicles, miRNA, microvesicles, peripheral nerve injury, hindlimb ischemia



## INTRODUCTION

Exosomes are types of extracellular vesicles (EVs) with diameters in the range of 30–150 nm, and as they take part in various biological processes and intercellular communication, several studies have appeared on this topic recently.<sup>1</sup> Trams et al. defined the term “exosome” in 1981 for the first time.<sup>2</sup> Subsequently, the International Society for Extracellular Vesicles (ISEV) has established a least evidence standard for extracellular vesicles (MISEV, 2018), which offers thorough guidelines for classifying and studying extracellular vesicles.<sup>3</sup> Three main types of EVs can be distinguished based on size and contents, biogenesis formation, and mechanisms: exosomes, microvesicles/ectosomes, and apoptotic bodies.<sup>4</sup> Dead cell fragments, with a size distribution from 500 to 800 nm, are known as apoptotic cells. Microvesicles originate directly from the budding of plasma cellular membranes and range in size from 200 to 1000 nm. The smallest EVs derived from endosomes with 30–150 nm diameter are called exosomes, in which different microenvironmental conditions can affect different amounts of exosome production and also various components at the cargo level.<sup>4,5</sup> Extensive studies have reported the critical role of exosomes in immune regulation, differentiation, apoptosis, angiogenesis through passing immunomodulatory cytokines, and noncoding RNAs (ncRNAs) like miRNAs between immune cells and other cell populations.<sup>6–8</sup> Notably, several ncRNAs can be transferred by exosomes including microRNA (miRNAs), long noncoding RNA (lncRNAs), and circular RNA (circRNAs), which are the

most attractive because of their role in intercellular communication.<sup>9</sup>

Exosomes for tissue repair engineering have many advantages over stem cells, including stability for a long period of time without affecting biological activity, the ability to target and repair organs quickly, and preserving bioactive components from digestion.<sup>10</sup> EVs can decrease incidence of iatrogenic tumor formation because of nonreplicable properties, reducing embolism formation accompanied by injection of mesenchymal stem cells (MSCs) and EVs resistant to damage by the hostile environment at the site of injury.<sup>11</sup> Research has been indicated that EVs derived from the epithelium, endothelium, neutrophils, platelets, mononuclear cells, and macrophages can improve treatment of neurodegenerative diseases.<sup>11–13</sup> A major problem in delivering exosomes is the quick clearance of the target organ. Therefore, it is imperative to develop formulation strategies that will enhance exosome retention time in target tissues.<sup>14</sup> In addition, there are various methods of exosome isolation, and each method of exosome isolation may affect the amount, type, and purity of exosomes.<sup>15,16</sup> Several studies have recommended that MSCs

**Received:** December 27, 2021

**Revised:** March 20, 2022

**Accepted:** March 31, 2022

**Published:** April 15, 2022



are suitable candidates for drug delivery through exosomes.<sup>17</sup> This review addresses the potential application of exosomes in neuronal tissue repair and how the choice of exosome donor cells can affect neuronal regenerative medicine.

### ■ IN VITRO APPLICATIONS OF EXOSOMES IN REGENERATIVE MEDICINE

Recent studies have suggested that the delivery of exosomal cargo can cause remarkable functional alterations in recipient cells.<sup>18</sup> However, isolation of exosomes from body fluids is possible, and the yield of exosomes is lower compared to that in cell culture media.<sup>15</sup> Hence, recognizing the origins of exosomes and isolating them from the extracellular matrix may also be a way to study them. Ferreira et al. found that the thrombin receptor activator peptide (TRAP-6) could stimulate higher production of exosomes from plate cells ( $8.6 \times 10^9$  exosomes versus  $4.2 \times 10^9$ ) without altering the average size of EVs. However, stimulation decreased the negatively charged phospholipid externalization in platelet-derived extracellular vesicles (PDEVs), which led to the decline in blood clotting activity compared with those obtained without agonist stimulation.<sup>19</sup> Several investigations have confirmed that exosomes facilitate the repair of myelin. MSCs derived from the placenta (PMSCs) are considered suitable cell sources to cure neurodegenerative diseases because they inhibit the killing of natural killer (NK) cells by expression of human leukocyte antigen-G (HLA-G) is a nonclassical MHC class I molecule on their surface. For example, Clark et al. discovered that EVs obtained from placenta-derived MSCs are effective at promoting myelin regeneration by inducing differentiation of endogenous oligodendrocyte precursor cells (OPCs) into mature myelinating oligodendrocytes.<sup>20</sup> Furthermore, Preußner et al. showed circRNAs as a novel class of noncoding RNAs present in all eukaryotic cells, especially in microvesicles and exosomes derived from platelets. Their result revealed that circRNAs, such as FAM13B, DYRK1A, AMD1, and TMEM30, observed high levels of expression in exosomes. Circular RNAs can transfer information from donor to recipient cells through selective release mechanisms. As the main cell target of platelets is reported to be endothelial cells, investigation on them can be used as prognostic and diagnostic biomarkers for inflammation and cancer metastasis.<sup>21</sup>

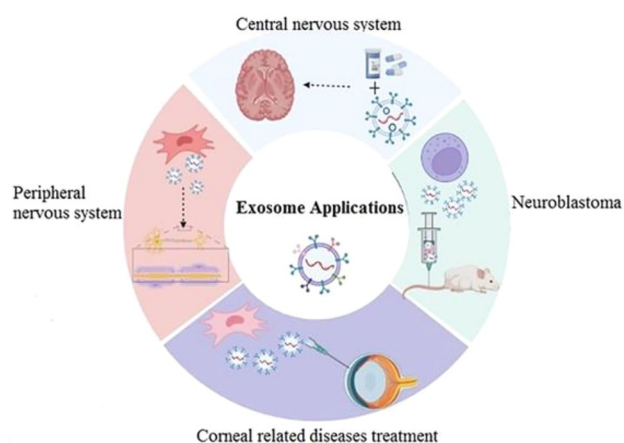
Treatment potential of exosomes has been demonstrated in research on fracture healing at different stages, suggesting that bone tissue repair can be facilitated using exosome delivery. Previously, Bouchareychas et al. discovered that exosomes produced by naive bone-marrow-derived macrophages (BMDM) contain anti-inflammatory miRNA-99a/146b/378a that is further enhanced by BMDM polarized by IL-4 (M2 macrophages).<sup>22</sup> These miRNAs could target NF- $\kappa$ B and TNF- $\alpha$  signaling so that BMDM exosomes stimulated with IL-4 may reveal a valuable therapeutic approach for atherosclerosis and other inflammatory diseases.<sup>22</sup> Furthermore, Wei et al. treated M1 macrophages and human bone marrow mesenchymal stem cells (hBMSCs) with exosomes isolated from differentiating bone-marrow-derived mesenchymal stem/stromal cells (BMSCs) at various stages of osteogenesis (day 0, day 3, day 7, and day 14). Their result showed that the expression of pro-inflammatory genes after treatment with 3 day exosomes and 7 day exosomes remarkably dwindled and exosomes at 3 days, 7 days, and 14 days led to a significant improvement in MSC migration as well as high expression of early osteogenic markers, such as alkaline phosphatase (ALP)

and bone morphogenetic protein 2 (BMP-2). Consequently, these results suggested that exosomes derived from differentiated MSCs have a unique function in osteoimmunomodulating bone dynamics.<sup>23</sup> Hydrogel formulations are appealing because they can be used as scaffolds, drug delivery vehicles, and cell encapsulation matrices. Compared to free molecules or cells, those incorporated inside hydrogels were found to be able to preserve their structures and functions for a long time.<sup>24</sup> Sun et al. loaded exosomes derived from macrophages in bioink hydrogel 10% (w/v) alginate (ALG) and 5% (w/v) hyaluronic with different concentrations of  $\beta$ -TCP acid with a 3D printing method. Their result indicated that the scaffold could release exosomes gradually during 3 days and enhance the osteogenic differentiation and immunosuppression as well as improve the angiogenic activity of cells in vitro.<sup>25</sup> Moreover, Liu et al. showed that macrophage M2-derived extracellular vesicles from THP-1 cells could enhance Young's modulus and improve osteogenic differentiation of bone marrow MSCs by increasing the expression of osteoblast differentiation markers (BMP2, BGLAP, COL1, and OSX) and increasing calcium nodule formation using biomimetic intrafibrillarly mineralized collagen scaffolds.<sup>26</sup> Hu et al. revealed that miR-23a-3p from hUC-MSCs-sEVs improved cartilage regeneration by activating the PTEN/AKT signaling pathway. They loaded exosomes in a Gelma/nanoclay hydrogel, and their result indicated desirable mechanical and biological performance accomplished by sustained release of sEVs ( $10 \times 10^8$  particles/mL). In other words, the Gelma/nanoclay/sEV hydrogel is a useful property for the treatment of cartilage defects.<sup>27</sup> In recent research, the potential role of exosomes in cardiovascular homeostasis and the cardiovascular system has been demonstrated. Zhu et al. used exosomes from adipose-derived stem cells (ASCs) for polarization of M1 to M2 to reduce inflammation and in mouse hindlimb ischemia (HLI) models and to stimulate endothelial cell proliferation, migration, and tube formation. Their result indicated exosomes derived from ASCs contain miR-21 as a positive marker for macrophage polarization.<sup>28</sup> Wang et al. suggested miR-126 from exosomes derived from endothelial progenitor cells (EPCs) could diminish acute injury and promote neurological function recovery after a stroke. Their result revealed that treatment of a diabetic mouse with EPC-exosomes led to decreased infarct size and increased cerebral blood flow (CBF) and cerebral microvascular density (MVD) with down-regulation cleaved caspase-3 on day 2 and vascular endothelial growth factor receptor 2 (VEGFR2) up-regulation until day 14.<sup>29</sup>

### ■ APPLICATIONS OF EXOSOMES IN NEURONAL TISSUES

Exosomes have been shown to have potential in treating neurodegenerative diseases. The neuroprotective and neuroregenerative features of exosomes are dependent on the donor cells and their activation state.<sup>11</sup> Exosomes derived from MSCs are useful candidates for cell-free therapies as they inherit the functions of MSCs, which can induce angiogenesis, anti-apoptosis, and anti-inflammatory effects. In addition, using natural agents with antioxidant activity improves neurogenesis. For instance, in animal models with multiple sclerosis, intranasal injections of exosomes loaded with curcumin to the microglia of the brain parenchyma gave rise to a functional recovery.<sup>4</sup> Furthermore, different cells including Schwann cells (SCs), microglia, oligodendrocytes, and astrocytes can release

exosomes for which their miRNA involves the nervous system (Figure 1). Maintenance and regeneration of peripheral nerve



**Figure 1.** Applications of exosomes as nanomaterials for neuronal regenerative medicine.

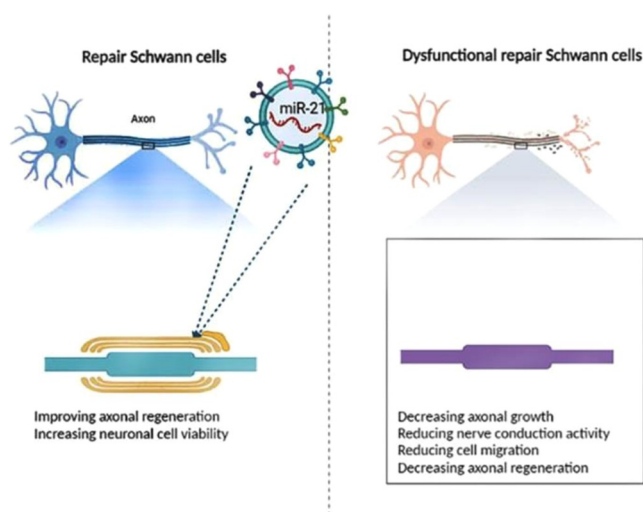
injury depends on the activities of SCs as the main peripheral nervous system glial cells.<sup>30</sup> Schwann-derived exosomes have also been confirmed to play a critical role in the peripheral and central nervous system.<sup>30,31</sup>

#### Applications of Exosomes in the Peripheral Nervous System

Peripheral nerve injury (PNI) has been a major health concern for decades, but current treatments are often ineffective, leading to poor recovery.<sup>32</sup> In terms of pathophysiology, several important factors are involved in nerve regeneration, such as macrophages and Schwann cells, inflammatory reactions, and vascular regeneration. Recent studies have shown that Schwann-derived exosomes enhance the regeneration process. Among the encapsulated myelin proteins found in exosomes, myelin-associated glycoprotein (MAG) and proteolipid protein (PLP) are involved in nerve remyelination. Moreover, researchers have recently discovered that miRNA encapsulated in exosomes including miR-132 contributes to the neural regulation of the brain through regulating the expression of vascular endothelial cadherin (VE-cadherin).<sup>32</sup>

Research confirmed that Schwann-derived exosomes could enhance axonal growth in vitro.<sup>32</sup> Simeoli et al. proposed that miR-21-5p was expressed in exosomes of dorsal root ganglia (DRG) sensory neurons following capsaicin treatment. Based on their findings, miR-21-5p was up-regulated in DRG neurons after peripheral axon injury and could reduce neuropathic hypersensitivity as well as inflammatory macrophage recruitment. Therefore, miR-21-5p from exosomes involves sensory neuron–macrophage communication after peripheral nerve damage.<sup>33</sup> SCs are remarkably plastic, allowing them to react in different situations to preserve the integrity of peripheral nerves.<sup>34</sup> Figure 2 demonstrates the delivery of exosomal miRNA-21 from repair Schwann cells improved axonal growth by down-regulation of the PTEN pathway and activation of PI3K in neurons, whereas dysfunctional repair of SC-derived exosomes prevents axonal growth and diminishes nerve conduction velocity.<sup>34</sup>

Studies suggested that electrical stimulation (ES) therapy may delay muscle atrophy in patients with hemiplegia and promote neurological function recovery and may benefit patients with diseases causing damage to the peripheral



**Figure 2.** Schematic representation of various phenotypic Schwann cells (mature myelinating, repair, and hypothetical dysfunctional repair SCs) releasing exosomes with different functions. Adapted with permission from ref 34. Copyright 2022 Wiley.

nervous system; however, the molecular mechanism is still unknown. Hu et al. used ES to stimulate nerve cells at 100 or 200 mV/mm for 0, 0.5, 1, and 2 h. Their result showed that coculturing nerve cells with Schwann cells could change neuronal activity by increasing glutamate levels and glutamate stimulating exosome secretion from Schwann cells, and they increased dorsal root axonal regeneration neurons in vitro and in vivo after nerve injury.<sup>35</sup> Despite being less studied, exosomes play an important role in SC-related diseases. Charcot–Marie–Tooth (CMT) disease is known as a typical hereditary neurological disorder of the PNS, in which its molecular mechanism that is associated with a mutation in an exosome biogenesis-related protein in SCs eradicated proper MVB formation and leads to a diminished number of exosomes and secretion of exosomal proteins and remyelination.<sup>34</sup> Guillain-Barré syndrome (GBS) is a disorder of the PNS that originates from this current notion of immunological attack, and M1-macrophage-derived exosomes have been connected with increased Th1 and Th17 immune responses.<sup>34</sup> Despite the fact that exosomes derived from SCs promote axonal regeneration, neuronal viability, and prevent apoptosis, many kinds of cancer are propagated by SCs, such as pancreatic cancer and melanoma.<sup>34</sup>

Furthermore, the PNS plays a crucial role in osteogenesis, bone metabolism, and bone remodeling. Wu et al. utilized SC-derived exosomes in a 3D-printed porous titanium alloy to enhance the efficacy of titanium alloy scaffolds in bone repair. Their result indicated that a combination of SC-derived exosomes with titanium alloy scaffolds could improve the proliferation, migration, and differentiation of BMSCs in vitro and in vivo during 12 weeks and could overcome biological limitations of titanium.<sup>36</sup>

#### NEUROBLASTOMA TREATMENT

Neuroblastoma (NB) is the most common extracranial tumor in children, and it is derived from neural crest stem cells during embryonic development.<sup>37</sup> Due to its highly heterogeneous molecular characteristics and consequent clinical outcome, NB treatment is, in most cases, difficult and diverse.<sup>37</sup> In this

context, regenerative medicine offers a higher therapeutic potential than currently used methods for NB treatment.

NB patients exhibit low miR-186 expression in NK cells which, together with NKG2D and DNAM-1 markers, can be considered a prognostic factor. Neviani et al. were able to inhibit tumor growth and spread by targeted delivery of NK-cell-derived exosomes carrying miR-186.<sup>38</sup> This treatment also acted in preventing the inhibition of NK cytotoxicity by targeting TGF $\beta$ .

NK-derived exosomes were also employed by Wang et al. in what they defined as a “cocktail therapy”, based on coating a nanoparticle with NK exosomes for tumor targeting.<sup>39</sup> This system showed high efficiency in targeting NB cells and delivering endogenous and exogenous miRNAs, such as let-7a, inside a dendrimer nanoparticle, leading to tumor growth suppression.<sup>39</sup> Moreover, miR-124 delivered by human adipose-derived mesenchymal stem cells (hADMSCs) to NB cell line was proven to have the potential to induce neuronal differentiation by repressing ELF4, transcription factor driving NB proliferation, and therefore decrease proliferation of cancer cells.<sup>40</sup>

In addition, PMSC-derived exosomes were tested on human NB cell line SH-SY5Y for their neuroprotective effect.<sup>41</sup> Medium conditioned with PMSC exosomes could reduce caspase activity in apoptotic cells and increase the number of neuronal projections. Kumar et al. were able to connect this result with the high expression of galactine-1 on the surface of PMSC exosomes (galactine-1 regulates neurites, cell adhesion, and cell proliferation in neurons).<sup>41</sup> These findings suggest a possible exosome-based treatment for neural tube defects during development, such as spina bifida.<sup>41</sup>

Furthermore, the transfer of molecular cargo in exosomes is important for cell–cell communication in a variety of cell types, including cancer cells. For several other tumor types, NB-derived exosomes promote growth and spread of the tumor. They transfer tumor-specific molecules to surrounding recipient cells by transporting their mRNA. Fonseka et al. found that the well-established oncogene N-Myc mRNA conferring chemoresistance could be carried by plasma exosomes in aggressive NB.<sup>42</sup> They also carry miRNAs such as miR199a-3p and miR375 that promote tumor proliferation and migration via regulating NEDD4 and PTEN expression, respectively. PTEN activates the PI3K/Akt signaling cascade and induces cell migration and proliferation, whereas NEDD4 is an onco-suppressor gene, pivotal in NB progression.<sup>43,44</sup> For these reasons, researchers suggested that tumor-derived exosomes become the target of new pharmaceutical studies for the inhibition of tumor spread.<sup>43,44</sup>

Lastly, exosomes can be utilized in NB diagnosis. Exosomes are optimal as noninvasive biomarkers for therapy monitoring and follow-ups. Villasante et al. found EZH2 and GLI1 mRNAs are carried in NB-derived exosomes.<sup>45</sup> The presence of those mRNAs could be used as pathological biomarkers for early prediction. Furthermore, exosome miRNAs were tested by Morini et al. in patients’ plasma before and after chemotherapy, and three miRNAs (miR-342-3p, -29c, let-7b) were identified for the difference in expression between good and poor responders to treatment.<sup>46</sup>

## ■ CORNEAL-RELATED DISEASE TREATMENT

The corneal tissue develops from neural crest stem cells (NCSCs) and still remains in adults a niche of postmigratory NCSCs. This tissue is interesting in several pathologies which

often leads to visual loss (through scars, haze, and opacities) that currently affects over 10 million people worldwide.<sup>47</sup> Corneal transplantation is the most common treatment for damaged cornea, but optical surgery is expensive and dangerous.<sup>47</sup> For this reason, regenerative medicine has developed strategies for less invasive actions through the use of mesenchymal stem cells and derived exosomes.

An important feature of corneal treatment is the correct and rapid tissue repair. The wound healing process was ameliorated by incubation of MSC-derived exosomes on the wounded cornea, resulting in healing acceleration of approximately +41.6%.<sup>48</sup> In 2020, a study by Wang et al. compared the efficacy in wound healing and regeneration of exosomes from MSCs and induced pluripotent stem cells.<sup>49</sup> While both exosomes exhibited an in vivo capacity for promoting regeneration and decreasing inflammation, iPSC exosomes had stronger effects.<sup>49</sup> In another study focused on diabetic limbal epithelial cells, limbal stromal cell (LSC)-derived exosomes promoted wound healing and up-regulation of LSC markers, keratin 15, and Frizzled-7.<sup>50</sup> Therefore, it can be suggested that exosomes from MSCs, iPSCs, and LSCs have been found to be suitable for treatment of ocular surface injuries to various degrees.

Scar tissue can represent a problem in cornea healing, as it impairs vision.<sup>51</sup> Shen et al. investigated the role of hADSC exosomes on corneal fibrosis.<sup>51</sup> Their findings suggested exosomal miRNA-19a suppressed keratinocyte differentiation in myofibroblasts by directly targeting kinase HIPK2, responsible for inducing pro-fibrotic protein production through the Smad3 pathway.<sup>51</sup> Exosomes from corneal stromal stem cells also seem to stimulate regeneration and prevent fibrotic scarring by decreasing fibrotic genes Col3a1 and Acta2 and blocking of neutrophil infiltration by miRNA transport.<sup>52</sup>

## ■ NEURAL CREST STEM CELLS: ORAL CAVITY

Neural crest stem cells are a group of stem, multipotent, embryonic cells forming transient structures during development. They result in the formation of the central nervous system (CNS), the peripheral nervous system (PNS), and craniofacial tissues by differentiating in several neuronal and non-neuronal cell types.<sup>53</sup> Nevertheless, postmigratory NCSC subgroups can still be found in NCSC-derived tissues in both children and adults, conserving the ability of self-renewal and differentiation potential.<sup>53</sup>

Postmigratory NCSCs are commonly found in the oral cavity. Dental pulp stem cells (DPSCs) are among the greatest hubs of NCSCs in the adult body.<sup>53</sup> Their exosome has been characterized by Hu et al., revealing high expression of superficial MSCs markers (CD73, CD90, CD166) and low levels of hematopoietic cell marker CD45.<sup>53</sup> Content analysis revealed a long list of miRNAs, among which miR-193a-5p involves immunomodulation. In the same work, the researchers focused on the different content of undifferentiated DPSCs and DPSCs which underwent odontogenic differentiation.<sup>53</sup> In differentiated DPSCs, exosomal miR-27a-5p promotes the up-regulation of the TGF $\beta$ 1 pathway by targeting the differentiation inhibitory molecule LTBP1, thus promoting the up-regulation of odontogenic genes (Runx2, ALP, DSP, DMP-1).<sup>53</sup> DPSC exosomes were also studied for their biological effect on various cell lines.<sup>54</sup> First, they exhibited a neuroprotective effect on degenerating hippocampal neurons by up-regulation of growth factor expression and activation of the endogenous apoptosis control pathway

through Bcl-2 expression control.<sup>54</sup> DPSC exosomes also induce apoptosis attenuation on neighboring cells in carries by promotion of Survivin and CL-Casp3 production.<sup>55</sup> Apoptosis was also reduced in an in vivo rat model of osteoarthritis through miR-140-5p-enriched DPSC exosomes by reduction of IL-1 $\beta$ -induced apoptosis.<sup>56</sup> A less recent study by Jarmalavičiūtė et al. also investigated the neuroprotective potential of DPSC exosomes by focusing on dopaminergic neurons during oxidative stress.<sup>57</sup>

Moreover, DPSC exosomes showed a role in immunomodulation.<sup>58</sup> They effectively suppressed inflammation in periodontitis through miR-1246 transport<sup>58</sup> and against ischemia reperfusion injury by down-regulation of TLR4, MyD88, and NF- $\kappa$ B inflammatory pathways.<sup>59</sup> Ji et al. investigated a comprehensive study on the main differences between bone marrow mesenchymal stem cells (BMMSCs) and DPSC-derived exosome immunomodulatory effects.<sup>60</sup> DPSC exosomes more effectively reduced the secretion of pro-inflammatory cytokines (IL-17), increased anti-inflammatory factor production (IL-10 and TGF $\beta$ ), and induced apoptosis of CD4<sup>+</sup> T cells.<sup>60</sup>

Furthermore, DPSC exosomes promote orofacial bone regeneration.<sup>61</sup> They can influence migration and differentiation of SCs toward odontoblasts during tooth development and regeneration.<sup>61</sup> They also have been explored as a MSC alternative in dental root filling in fibrine-based substrates.<sup>62</sup> A previous study of periodontitis showed that DPSC exosomes suppressed alveolar bone resorption by attenuating bone loss in vivo.<sup>63</sup> Swanson et al. engineered a biodegradable polymer delivery platform for sustained release of exosomes in craniofacial bone regeneration. In all of these works, exosomes have led to tissue mineralization and bone healing.<sup>64</sup>

Another lesser explored source of postmigratory NCSCs in the oral cavity is the periodontal ligament.<sup>65</sup> Periodontal ligament stem cells (PLSCs) produce exosomes carrying several miRNAs (miR-24-2, miR-142, miR-335, miR-490, and miR-296) which might be involved in oncogene silencing.<sup>65</sup> They also exhibit biological effects in inducing de/inflammation of tissues.<sup>66</sup> Through NF- $\kappa$ B reporter assays Cebatariūnienė et al. demonstrated that PLSC exosomes suppress inflammation and NF- $\kappa$ B activity through the PI3K/Akt signaling pathway and could be used to tackle chronic inflammation in periodontitis.<sup>67</sup> In addition, they promote osteogenesis and osteogenic differentiation on BMSCs through regulation of MAPK and AMPK signaling pathways.<sup>66</sup> Bone regeneration is strongly associated with angiogenesis.<sup>68</sup> A study on rat calvaria defects confirmed that PLSC-derived exosomes strongly promote vascularization, together with osteogenesis, through vascular endothelial growth factor (VEGF) expression.<sup>69</sup>

Lastly, periapical cyst-MSCs also express NCSC markers and can differentiate in functional neurons. In the future, periapical cyst stem-cell-derived exosomes may be of interest for neuroprotection and neuroregeneration, even though they are not well studied yet.<sup>68</sup>

## ■ NEURAL CREST STEM CELLS: BONE MARROW

Bone marrow stromal cells contain, among them, stem cells presenting NCSC markers.<sup>70</sup> This subpopulation's exosomes have important applications in the neuro field.<sup>70</sup> First, BMSC characteristics and content have been thoroughly investigated. El-Derany et al. found several exosome miRNAs (miR-21-5p,

miR-125b-5p, miR-199a-3p, miR-24-3p, let-7a-5p) with a neuropathy feature.<sup>71</sup> According to Xiao et al. found BMSC exosomes also carry miRNA-134, which targets caspase-8, an initiator caspase, in oligodendrocytes, therefore inhibiting endogenous induced apoptosis.<sup>72</sup> MiRNA-544 was also found to have therapeutic, anti-inflammatory properties on spinal cord injuries.<sup>73</sup> This immunomodulatory effect was later confirmed by in vivo studies on spinal cord rat models.<sup>74,75</sup> Jia et al. indicated intravenous injections of exosomes secreted by BMSCs in the rats suffering from spinal cord injuries played a vital role in the healing process by increasing expression of the repair protein sonic hedgehog (Sh).<sup>74</sup> Moreover, Zhou et al. showed that, in rats that received the 200  $\mu$ L of exosomes via the tail vein, there was a reduction in neuronal cell death, an improvement in myelin arrangement, and a reduction in myelin loss, as well as an increase in pericyte/endothelial cell coverage of vascular walls.<sup>75</sup>

BMSC exosomes also have a role in the anti-inflammatory response, as demonstrated by Yuan et al.<sup>76</sup> BMSC exosomes induce autophagy in microglial cells through miR-32 regulation of DAB2IP.<sup>76</sup> Other studies linked BMSC exosomes to M2 polarization of microglia, confirming their immunomodulation effect.<sup>77</sup>

Consequently, BMSC exosomes exhibit a neuroprotective effect.<sup>78</sup> For instance, BMSC-derived exosomes containing ubiquitin proteasome and histones could increase the level of in vitro and in vivo neuronal differentiation and promote neuron recovery in a Parkinson's disease (PD) rat model.<sup>78</sup> Another feature of BMSC exosome cargo is the promotion of synoviocyte activation through the miR-143-3p targeting NF- $\kappa$ B pathway, important for arthritis treatment.<sup>79</sup> BMSC exosomes also may play a role in angiogenesis stimulation during bone regeneration.<sup>80</sup> Guo et al. revealed a miR-206-3p expression in orofacial mesenchymal stem cell (OMSC)-derived exosomes targeting bone morphogenetic protein-3 (Bmp3) and nuclear factor of activated T-cells, the cytoplasmic one (NFATc1), that may regulate orofacial bone development and establish the balance between osteoblasts and osteoclasts that is essential for orofacial bone formation.<sup>81</sup> In addition to application of natural BMSC exosomes, exosomes enriched with Zeb2/Axin2 proteins are also used to achieve improvement in poststroke neuroplasticity and functional recovery in rat models.<sup>82</sup>

## ■ CHARACTERISTICS OF EXOSOMES USED IN NEURAL FIELDS

In the past few years, several studies have reported the potential application and role of exosomes in central neural development and regeneration. EVs are released by neurons and glia cells in the central nervous system and mesenchymal stem/stromal cells under physiological and pathological conditions.<sup>83</sup>

In recent years, attention has focused on the numerous advantages and potential of exosomes, as much so that the term "nanotreatments" has been introduced, particularly in the neurological field.<sup>83</sup>

Among the advantages mentioned above, there is the smaller size of exosomes, longer shelf life, and fewer side effects. Scientists have been able to establish the beneficial role of exosomes in several neurodegenerative disorders including Parkinson's disease, neuritis, encephalomyelitis (EAE), Alzheimer's disease, cerebral ischemia, etc.<sup>83,84</sup>

Several studies have recommended that MSCs are suitable candidates for drug delivery through exosomes, and exosomes made from MSCs may provide considerable advantages.<sup>83,85</sup> For these reasons, Riazifar et al. have proposed to use MSC-derived exosomes as cell-free therapies to treat autoimmune and central nervous system disorders.<sup>83</sup> Exosomes produced by MSCs, which were stimulated by IFN $\gamma$ , were found to lower the mean clinical score of EAE mice, decrease demyelination, and lower neuroinflammation when they were given intravenously.<sup>83</sup> Shiue et al. have investigated in rats the effect of mesenchymal stem cell exosomes as a cell-free therapy for nerve injury.<sup>85</sup> They found exosome infusion demonstrated excellent prevention and reversal of nerve ligation-induced pain.<sup>85</sup> This could be a novel therapeutic approach for this type of nerve disease.<sup>85</sup> Therefore, it has been demonstrated that cell-derived exosomes can act as biological nanoparticles with positive effects in different pathological conditions, including Parkinson's disease.<sup>84</sup> Vilaça-Faria et al. have shown that exosomes act as modulators in the neuroregeneration process, which is fundamental for the blockage of progression of PD.<sup>84</sup> Therefore, many neurodegenerative diseases, including Parkinson's disease, could be treated with MSC secretomes.

Adipose-derived exosomes (ADSCs) have been observed to have therapeutic potential for treatment of neurodegenerative disorders.<sup>86</sup> In 2016, Lee et al. found a role of ADSC-derived exosomes in the treatment of Huntington's disease (HD), which is a form of neurodegenerative disease caused by the accumulation of mutant Huntingtin (mHtt).<sup>86</sup> They discovered ADSC secretion of various neurotrophic factors and microvesicles, which affects the disease's unfolding proteins through paracrine mechanisms.<sup>86</sup> The therapeutic role of exosomes from ADSCs is given by their ability to significantly decrease mHtt aggregates in R6/2 mice-derived neuronal cells.<sup>86</sup> Later, in 2018, Lee et al. proposed a correlation between the quantity of exosomes from adipose-derived stem cells and the level of  $\beta$ -amyloid ( $A\beta$ ) in the brain.<sup>87</sup> Extracellular vesicles secreted from ADSCs can reduce cellular apoptosis and  $A\beta$  pathology by carrying some beneficial components to the cells.<sup>87</sup>

The effects of adipose-derived stem cell exosomes have been evaluated also in amyotrophic lateral sclerosis (ALS), an inherited condition characterized by the death of motor neurons in the cortex, brain stem, and spinal cord.<sup>88</sup> The capability of modulation and modification of an adverse microenvironment is due to the paracrine effect of extracellular vesicles.<sup>88</sup> A study from 2016 demonstrated that ADSC-exos modulate cellular phenotypes of ALS, alleviating aggregation of superoxide dismutase 1 (SOD1) and therefore can be considered as a potential therapeutic candidate for ALS.<sup>88</sup> The exosome markers isolated from ADSC are the same as the most common ones of MSC, such as CD9, CD63, and HSP70.<sup>31,89</sup>

In addition to ADSCs, several other exosome types have been exploited for therapeutic purposes in the neural field from glial cell lines, particularly Schwann cells.<sup>89</sup> In 2020, Wang et al. demonstrated for the first time that the systemic administration of exosomes derived from healthy Schwann cells (SC-Exos) to diabetic mice with diabetic peripheral neuropathy (DPN) significantly ameliorated this disease.<sup>89</sup> It is due to the interaction between Schwann cell exosomes, containing different miRNAs (miR-21, -27a, and -146a) and their target proteins in sciatic nerve tissues.<sup>89</sup> This reduces axonal and myelin damage of the sciatic nerve in the treatment

of DPN.<sup>89</sup> Another investigation over SC-derived exosomes reported them as a novel therapeutic strategy for nervous system injury.<sup>31</sup> SC-derived exosomes can modulate the damaged PNS microenvironment and enhance axonal regeneration by inhibiting the activity of the GTPase RhoA.<sup>31</sup> Another research group investigated the role of exosomes released by endogenous Schwann cells on neurite outgrowth and axon regeneration.<sup>90</sup> SC-derived exosome markers have been expressed in common markers including Alix, CD9, CD63, and HSP70.<sup>90</sup>

Another type of glial cell involved in preservation and maintenance of neural axons are oligodendrocytes via producing extracellular vesicles with contribution to overall neural homeostasis.<sup>91</sup> This effect is due to delivery of biomolecules including nucleic acids or energy substrates to target cells, which are able to exert a modulation of the tissue microenvironment and sustain axonal projections.<sup>91</sup>

Properties and characteristics of key proteins transported from extracellular vesicles have been studied also in Parkinson's disease as one of the most common neurodegenerative disorders.<sup>92</sup> Yu et al. in 2020 found a correlation between the level of exosomes secreted by oligodendrocyte and the progression of disease.<sup>92</sup> They observed that EVs derived from that cell contain higher levels of  $\alpha$ -synuclein ( $\alpha$ -syn), and  $\alpha$ -syn aggregation is directly related to progression of Parkinson's disease.<sup>92</sup> Therefore, oligodendrocyte-derived enriched microvesicles (OEMVs) could be a potential biomarker for multiple system atrophy (MSA).

Accumulation of  $\alpha$ -syn was also investigated in microglial exosomes. Extracellular vesicles released by microglia cause protein aggregation in nearby neurons, contributing to the progression of pathology.<sup>93</sup>

A greater number of markers were identified for oligodendrocyte-derived exosomes as compared to Schwann or microglial cells. In fact, in addition to those already mentioned above, CD81, oligodendroglial proteolipid protein (PLP), 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), and Sirtuin-2 (Sirt-2) as well as common markers Tsg101 and Flotillin-1 (Flot-1) were also identified.<sup>91,92</sup>

Macrophages/microglia divide two different groups based on their phenotypes, the pro-inflammatory classically activated (M1) and the anti-inflammatory alternatively activated (M2) phenotype.<sup>93</sup> In some cases, the nature of the vesicles and their material transport capacity has been exploited as drug carriers. Recently, Gao et al. used M2-type primary peritoneal macrophage exosomes for transport of berberine (Ber), as an anti-inflammatory and neuroprotective pharmaceutical compound, with a loading efficiency 17% and sustained release of 71% during 48 h through the blood-brain barrier (BBB) for spinal cord injury therapy.<sup>94</sup> Because of the natural benefits of exosomes, it was possible to administer medicine and bypass the BBB barrier; it could be a possible safety protocol for administering drugs to patients.<sup>93,94</sup>

Recent studies have suggested macrophages contribute to the development of neuroinflammation in the peripheral nervous system or central nervous system by secretion of cytokines. It is known that M1 phenotype macrophages are the key factor in inflammation, so in some situations, the extracellular vesicles secreted by cells, in this case macrophages, could have a negative effect. In fact, scientists have demonstrated that exosomes released by M1 macrophages cause experimental autoimmune neuritis.<sup>95</sup>

In contrast, macrophages that are appropriately stimulated and induced into M2 phenotypes have anti-inflammatory effects. This has been demonstrated through the use of exosomes produced from lipopolysaccharide (LPS)-stimulated macrophages that are able to stimulate microglial polarization from the M1 phenotype to the M2 phenotype, inhibiting the inflammation response, with protective and beneficial effects in neuronal diseases.<sup>96</sup>

The protective role of exosomes derived from cells have been investigated also in endothelial cells; for example, in the central nervous system, microvascular vessels are mainly composed of endothelial cells (ECs), and after damage such as ischemic stroke, the ECs could stimulate angiogenesis and migration of neural progenitor cells (NPCs), thus contributing to neurovascular unit repair and tissue remodeling. This process is mediated by exosomes produced by endothelial cells; in fact, Zhou et al. demonstrated that EC-exo treatment increased NPC survival and improved neurological outcomes.<sup>96</sup> As a result, EC-derived exosome therapy was shown to have beneficial effects during ischemic stroke because of their brain-protective properties.<sup>96</sup>

In another study of 2017, the authors proposed that EC-derived exosomes contribute to protective effects from neurological damage during ischemia/reperfusion injury (I/R) in femoral arteries. The cells under study were human umbilical vein endothelial cells (HUVECs) and the exosomes secreted by them. Furthermore, the protective role of cell-derived exosomes was confirmed toward nerve cells against I/R injury by promoting migration, invasion, and cell growth.<sup>97</sup>

### ■ EXOSOMES FOR TREATMENT OF BRAIN STROKE

There are over 13.7 million new strokes each year, and with these numbers, stroke is still the third cause of death worldwide.<sup>98</sup> Stroke events are caused by a partial disruption of cerebral blood flow to the brain, and the related injuries are associated with high morbidity and mortality.<sup>98</sup> For this disease as for many others, exosomes can play an important role to induce a regenerative process after a stroke.<sup>98</sup> They can transport miRNA and other mediators among several types of brain cells, leading to injury healing.<sup>98</sup> Currently, very few therapeutic drugs are capable of crossing the BBB, and there is no effective strategy for treating stroke, but exosomes are able to cross the BBB and are a promising therapy for stroke.<sup>98</sup> Many research groups performed different treatments with exosomes. Jiang et al. found that exosomes enriched with an anti-inflammatory miRNA (miR-30d-5p), as treatment for patients with acute ischemic stroke (AIS) have a protective effect.<sup>98</sup> They conducted both *in vitro* and *in vivo* studies, demonstrating that exosomes thus functionalized could induce macrophages to take on an M2 phenotype with anti-inflammatory properties in an area of cerebral injury.<sup>98</sup>

Ling et al. observed that intravenous injection of exosomes from urine stem cells (USCs) enhanced neurogenesis and alleviated neurological deficits in postischemic stroke rats.<sup>99</sup> William et al. evaluated the effect of MSC-derived exosomes on swine models after traumatic brain injury (TBI) and hemorrhagic shock.<sup>100</sup> RNA sequencing analysis was performed on the brain tissue after death. The results showed an increase in the expression of all genes related to neurogenesis, neuronal development, and synaptogenesis and clear reduction in the expression of genes related to stroke, neuroinflammation, neuroepithelial cell proliferation, and non-neuronal cell proliferation contributing to reactive gliosis.<sup>100</sup> Moreover,

Zhang et al. examined the effect of derived transplanted stem cell exosomes as a therapy for ischemic stroke. In both *in vivo* and *in vitro* experiments, they found that these types of exosomes were able to reach the site of ischemic damage and induce microglia-mediated inflammation that was attenuated after oxygen glucose deprivation *in vitro* and significantly reduced the infarct volume *in vivo*, improving behavioral deficits in mice.<sup>101</sup> Exosomes are able to attenuate different damage after stroke, as demonstrated by Nalamolu et al.'s group.<sup>102</sup> They found that exosomes isolated from coculture of regular and oxygen-glucose-deprived MSCs attenuated the poststroke brain damage and improved the neurological outcome in rat models.<sup>102</sup> In addition to MSC-derived exosomes, Jiang et al. understood that also plasma exosomes (Pla-Exo) provide protection against I/R injury by a HSP70 molecular pathway.<sup>103</sup> Furthermore, Zhang et al. worked on exosomes from adipose mesenchymal stem cells (AMSCs). This group found that miR-22-3p contained in exosomes isolated from AMSCs could reduce cerebral I/R injury, one of the most common types of stroke, through modulation of the KDM6B/BMP2/BMF molecular pathway.<sup>104</sup>

Another way to use the exosomes as a treatment for stroke is to use them as a carrier for drug delivery. Guo et al., for example, loaded plasma exosomes (EXO) with Edaravone, a known drug with neuroprotective effects, to improve the outcomes for stroke treatment.<sup>105</sup> Other groups focused on functionalizing the exosomal surface, for example, to improve the capability of stroke drugs to penetrate the BBB.<sup>105</sup>

### ■ EXOSOME FOR TREATMENT OF MULTIPLE SCLEROSIS

Currently, 2.5 million people worldwide suffer from multiple sclerosis (MS).<sup>106</sup> Symptoms include neuroaxonal degeneration in the central nervous system, resulting from an autoimmune attack on the brain and spinal cord.<sup>106</sup> Today it is known that exosomes have a therapeutic potential in MS, and with this knowledge, many groups set up a lot of studies.<sup>106</sup> Baharloo et al. evaluated the suppressive efficacy of umbilical cord mesenchymal stem cells (UC-MSCs) and their exosomes on the proliferation of peripheral mononuclear blood cells (PMBCs) in relapsing–remitting multiple sclerosis (RRMS) patients and healthy subjects.<sup>106</sup> It is obvious that MSCs inhibit proliferation of autoreactive lymphocytes involving in MS pathogenesis. They established that human UC-MSC-derived exosomes could mimic therapeutic efficiency of their parental cells and effectively suppress proliferation of the PMBCs in both RRMS patients and healthy controls.<sup>106</sup> MS is an autoimmune disease and pathogenic T lymphocytes play an important role in disease development. Azimi et al. studied the effects of T regulatory exosomes on the proliferation or survival of T lymphocytes from RRMS patients and healthy controls.<sup>107</sup> This study was motivated by a validated concept that impaired function of regulatory T cells (Tregs) is a pathogenic mechanism for MS. Regulatory T cells derived from MS patients and healthy controls were cultured for 3 days. Exosomes were isolated from supernatants, and Treg-derived exosomes were cocultured with conventional T cells (Tconv).<sup>107</sup> Treg-derived exosomes suppressed Tconv proliferation in MS patients compared to that in healthy controls. Their research found that the expression of particular pathogenic miRNA (miR-326) in T-cell-derived exosomes of RRMS patients resulted in down-regulation compared to the healthy control. Also T-cell-derived exosome miRNAs can be

considered potential targets for the treatment of MS.<sup>107</sup> A similar study was performed by Kimura et al.<sup>108</sup> They found that miRNA let-7i contained in circulating exosomes regulates MS pathogenesis by blocking the IGF1R/TGFBR1 pathway.<sup>108</sup> This event occurs through reduction of the frequency of the functional Treg cells via inhibition of their differentiation from naive CD4<sup>+</sup> T cells.<sup>108</sup>

Additionally to T lymphocytes, B lymphocytes play a crucial role in the pathogenesis of MS. Recently, Benjamins et al. focused on B cell exosome-enriched (Ex-En) fractions.<sup>109</sup> They tested conditioned medium (Sup) from cultures of B cells from blood of MS patients without treatment to see if it harmed neurons and oligodendrocytes. Their result showed that exosomes are responsible for much of the in vitro toxicity on oligodendrocytes from MS patients' B cells compared to the control group.<sup>109</sup> The effect of BMSC exosomes on microglial polarization has been studied in relation to MS progression.<sup>110</sup> Li et al. demonstrated that BMSC exosome-treated autoimmune encephalomyelitis (EAE) rats showed an increase in M2 polarization, production of anti-inflammatory cytokines (IL-10, TGF $\beta$ ), and a decrease in pro-inflammatory cytokines (TNF- $\alpha$  and IL-12).<sup>110</sup> Another recent study discussed the effect of BMSC exosomes in the EAE model and compared to the cuprizone diet model as a toxic demyelination model.<sup>70</sup> Their result indicated MSC-Exo can cross the BBB and reach neural cells and could reduce neuroinflammation through the NF- $\kappa$ B pathway inhibition and reduce amyloid- $\beta$  precursor protein density.<sup>70</sup> This led to oligodendrocyte proliferation, maturation, and remyelination.<sup>70</sup>

### ■ EXOSOMES FOR TREATMENT OF PARKINSON'S DISEASE

More than 10 million people worldwide are suffering from Parkinson's disease. Parkinson's disease is a neurodegenerative disorder that affects predominately dopamine-producing ("dopaminergic") neurons in a specific area of the brain called substantia nigra.<sup>111</sup> As a result, this pathology leads to motor and cognitive dysfunction. Exosomes show a therapeutic potential in this neurodegenerative disease, and for this reason much research has emerged in recent years.<sup>111</sup>

Kojima et al. developed EXOTic devices enabling efficient customizable production of designer exosomes. These EXOTic acts as a treatment of Parkinson's disease by the delivery of catalase mRNA into the brain.<sup>111</sup> They found that therapeutic-specific catalase mRNA delivery by designer exosomes attenuated neurotoxicity and neuroinflammation in the in vitro and in vivo models of Parkinson's disease.<sup>111</sup> Cheng et al. analyzed the effect of exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs) in PD.<sup>112</sup> They observed that a pretreatment with exosome-promoted 6-OHDA-stimulated SH-SY5Y cells proliferated and inhibited apoptosis by inducing autophagy.<sup>112</sup> Moreover, they detected in vivo on the rat model that exosomes reached the substantia nigra through the BBB, relieved apomorphine-induced asymmetric rotation, reduced substantia nigra dopaminergic neuron loss and apoptosis, and up-regulated the level of dopamine in the striatum.<sup>112</sup> Similar to other neurodegenerative diseases, PD is challenging to treat, largely because the BBB blocks passage of most drugs.<sup>113</sup> With this awareness, Qu et al. observed the brain-targeting ability of blood exosomes and that these exosomes can be used as a promising drug delivery platform for targeted therapy against PD.<sup>113</sup> PD is a disease characterized by a lack of dopamine. For this reason,

they tested dopamine-loaded exosomes to observe a better therapeutic efficacy in a PD mouse model.<sup>113</sup> Furthermore, Liu et al. showed that functionalization of the exosome surface and its content was used as a nanoscavenger for clearing  $\alpha$ -synuclein aggregates and reducing their cytotoxicity in Parkinson's disease neurons.<sup>114</sup> In addition, the potential use of exosomes as carriers is well-known today. Exploiting the exosome's ability, Ren et al. demonstrated the therapeutic potential of functionalized exosomes with a neuron-specific rabies viral glycoprotein to deliver a DNA aptamer capable of targeting  $\alpha$ -synuclein aggregates that are the main component of Lewy bodies in Parkinson's disease brain.<sup>115</sup> Blood exosomes already mentioned can be used as direct treatment, as confirmed by Sun et al.<sup>116</sup> They found that blood-derived exosomes from healthy volunteers alleviated impaired motor coordination in a mouse model of PD. Results from immunohistochemistry and Western blotting indicated that the loss of dopaminergic neurons in substantia nigra and striatum of PD model mice was rescued by the exosome treatment.<sup>116</sup> This treatment also restored the homeostasis of oxidative stress, neuroinflammation, and cell apoptosis in the model mice of PD.<sup>116</sup> Li et al. found that miR-188-3p plays a key role in modulating PD. Their data suggested that miR-188-3p-overexpressed exosomes had therapeutic effects on PD by suppressing the expression of autophagy and inflammasomes via targeting NALP3 and CDK5, both in the cell model and in the mice model.<sup>117</sup>

### ■ EXOSOMES FOR TREATMENT OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a neurodegenerative disease, and it is the most common cause of dementia. AD is characterized by abnormal amounts of amyloid-beta ( $A\beta$ ), accumulating extracellularly as amyloid plaques, and tau proteins, accumulating intracellularly as neurofibrillary tangles. This condition in the brain that affects neuronal functioning and connectivity leads to a progressive loss of brain function. Moreover, this complex neurodegenerative disorder has no definite treatment.<sup>118</sup>

Also for AD, exosomes are studied as possible carriers to deliver specific drugs or therapeutic molecules. Wang et al., for example, used exosomes to carry curcumin beyond the BBB. They demonstrated that curcumin inhibited tau phosphorylation, a characteristic event of AD, improving the outcome of the disease.<sup>118</sup> Huo et al. considered using exosomes like cargo for defined drugs for AD to improve their delivery beyond the BBB. In this work, they enhance the delivery of silibinin using macrophage-derived exosomes. The dual action of silibinin, reducing  $A\beta$  aggregation and deactivating astrocytes, to improve behavior and cognitive performance in subjects with AD was increased.<sup>118</sup> Moreover, Qi et al. used exosomes like carriers delivering quercetin-loaded exosomes to the brain in the AD mice model.<sup>119</sup> The treatment of exosomes containing quercetin improved cognitive function by inhibiting phosphorylated tau-mediated neurofibrillary tangles.<sup>119</sup>

MSC-derived exosomes have been demonstrated to prevent memory deficits in the animal model of AD. Chen et al. wanted to evaluate MSC-derived exosomes on both in vitro and in vivo models of AD. Their results showed that MSC exosomes reduced  $A\beta$  expression and restored the expression of neuronal memory/synaptic plasticity-related genes in both the cell model and the mice model.<sup>120</sup> These data were also confirmed by the work of Wang et al., who saw that exosomes are able to



improve cognitive function recovery in AD mice by activating the sphingosine kinase/sphingosine-1-phosphate signaling pathway.<sup>121</sup> Another group showed that intracerebroventricularly injected BM-MSCs through their exosome improved cognitive impairment in AD model mice by ameliorating astrocytic inflammation as well as synaptogenesis. They especially observed that an increasing expression of miR-146 in the hippocampus facilitates improved cognitive functions.<sup>122</sup>

It is now known that exosomes contain miRNA, which can act at multiple levels in AD. Related to this topic, Jahangard et al. found that mir-29b is significantly reduced in AD, suggesting a role in the pathogenesis of the disease. With this observation, they decided to make an injection of exosomes packed with miR-29b in a mice model, and they demonstrated that this treatment has protective effects against amyloid pathogenesis.<sup>123</sup> A similar study was done by Zhai et al. They used ADMSCs that were transfected into the miRNA-22 mimic to obtain miRNA-22-loaded exosomes (Exo-miRNA-22), which were further used for the treatment and nerve repair in the AD mice model. As a result, the survival level of nerve cells in mice was higher in the Exo-miRNA-22 group, and the expression of inflammatory factors was lower than that in the control group, indicating Exo-miRNA-22 could significantly suppress neuroinflammation.<sup>124</sup> As already mentioned, a way to improve the treatment with the exosomes is to functionalize their surface. Cui et al., for example, used a CNS-specific rabies viral glycoprotein peptide to target intravenously infused exosomes derived from MSCs (MSC-Exo) to the brain of transgenic APP/PS1 mice. As a result, they obtained a significant improvement in learning and memory capabilities with reduced plaque deposition and A $\beta$  levels and normalized levels of inflammatory cytokines.<sup>125</sup> AD is a complex disease that results from the interaction of many components. One of these components is P-glycoprotein (P-gp), a member of the ABC transporter family situated in the BBB that plays a role on cleaning the A $\beta$  accumulation. P-gp in a pathological BBB was lower than that in a normal BBB, thus impeding the clearance of A $\beta$ , and so it can be important in AD development. With this knowledge, Pan et al. used human brain microvascular endothelial cell-derived exosomes containing P-gp up-regulated as an extracorporeal A $\beta$  cleaning system to remove A $\beta$  peptides from the brain to obtain potentially ameliorated cognitive dysfunction in AD mice.<sup>126</sup>

## AUTHOR INFORMATION

### Corresponding Author

**Barbara Zavan** – Department of Translational Medicine, University of Ferrara, 44121 Ferrara, Italy; [orcid.org/0000-0002-4779-4456](https://orcid.org/0000-0002-4779-4456); Email: [barbara.zavan@unife.it](mailto:barbara.zavan@unife.it)

### Authors

**Elham Pishavar** – Department of Translational Medicine, University of Ferrara, 44121 Ferrara, Italy

**Martina Trentini** – Department of Translational Medicine, University of Ferrara, 44121 Ferrara, Italy

**Federica Zanotti** – Department of Translational Medicine, University of Ferrara, 44121 Ferrara, Italy

**Francesca Camponogara** – Department of Translational Medicine, University of Ferrara, 44121 Ferrara, Italy

**Elena Tiengo** – Department of Translational Medicine, University of Ferrara, 44121 Ferrara, Italy

**Iliaria Zanolla** – Department of Medical Science, University of Ferrara, 44121 Ferrara, Italy

**Massimo Bonora** – Department of Medical Science, University of Ferrara, 44121 Ferrara, Italy

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsnanoscienceau.1c00062>

### Author Contributions

E.P., M.T., F.Z., F.C., I.Z. wrote the review; E.T. formatted the literature search; M.B. revised the paper; B.Z. supervised the work.

### Notes

The authors declare no competing financial interest.

## REFERENCES

- (1) Alenquer, M.; Amorim, M. J. Exosome biogenesis, regulation, and function in viral infection. *Viruses* **2015**, *7* (9), 5066–5083.
- (2) Trams, E. G.; Lauter, C. J.; Salem, N., Jr.; Heine, U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **1981**, *645* (1), 63–70.
- (3) Théry, C.; Witwer, K. W.; Aikawa, E.; Alcaraz, M. J.; Anderson, J. D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G. K.; et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *Journal of extracellular vesicles* **2018**, *7* (1), 1535750.
- (4) Xie, M.; Wu, D.; Li, G.; Yang, J.; Zhang, Y. S. Exosomes targeted towards applications in regenerative medicine. *Nano Select* **2021**, *2* (5), 880–908.
- (5) Achreja, A.; Meurs, N.; Nagrath, D. Quantifying metabolic transfer mediated by extracellular vesicles using Exo-MFA: an integrated empirical and computational platform. *Metabolic Flux Analysis in Eukaryotic Cells*; Springer, 2020; pp 205–221.
- (6) Kalantari Khandani, N.; Ghahremanloo, A.; Hashemy, S. I. Role of tumor microenvironment in the regulation of PD-L1: A novel role in resistance to cancer immunotherapy. *Journal of cellular physiology* **2020**, *235* (10), 6496–6506.
- (7) Ni, Y.-Q.; Lin, X.; Zhan, J.-K.; Liu, Y.-S. Roles and functions of exosomal non-coding RNAs in vascular aging. *Aging and disease* **2020**, *11* (1), 164.
- (8) Cai, M.; Shi, Y.; Zheng, T.; Hu, S.; Du, K.; Ren, A.; Jia, X.; Chen, S.; Wang, J.; Lai, S. Mammary epithelial cell derived exosomal MiR-221 mediates M1 macrophage polarization via SOCS1/STATs to promote inflammatory response. *International immunopharmacology* **2020**, *83*, 106493.
- (9) Li, C.; Ni, Y.-Q.; Xu, H.; Xiang, Q.-Y.; Zhao, Y.; Zhan, J.-K.; He, J.-Y.; Li, S.; Liu, Y.-S. Roles and mechanisms of exosomal non-coding RNAs in human health and diseases. *Signal transduction and targeted therapy* **2021**, *6* (1), 383.
- (10) Kona, V. B. R.; Mamidi, M. K.; Bhone, R.; Das, A. K.; Pochampally, R.; Pal, R. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. *Cytotherapy* **2016**, *18* (1), 13–24.
- (11) Sidoryk-Węgrzynowicz, M.; Dąbrowska-Bouta, B.; Sulkowski, G.; Strużyńska, L. Nanosystems and exosomes as future approaches in treating multiple sclerosis. *European Journal of Neuroscience* **2021**, *54* (9), 7377–7404.
- (12) Wang, P.; Wang, H.; Huang, Q.; Peng, C.; Yao, L.; Chen, H.; Qiu, Z.; Wu, Y.; Wang, L.; Chen, W. Exosomes from M1-polarized macrophages enhance paclitaxel antitumor activity by activating macrophages-mediated inflammation. *Theranostics* **2019**, *9* (6), 1714.
- (13) Lombardi, M.; Parolisi, R.; Scaroni, F.; Bonfanti, E.; Gualerzi, A.; Gabrielli, M.; Kerlero de Rosbo, N.; Uccelli, A.; Giussani, P.; Viani, P.; et al. Detrimental and protective action of microglial extracellular vesicles on myelin lesions: astrocyte involvement in remyelination failure. *Acta neuropathologica* **2019**, *138* (6), 987–1012.
- (14) Yamashita, T.; Takahashi, Y.; Takakura, Y. Possibility of exosome-based therapeutics and challenges in production of

- exosomes eligible for therapeutic application. *Biol. Pharm. Bull.* **2018**, *41* (6), 835–842.
- (15) Perocheau, D.; Touramanidou, L.; Gurung, S.; Gissen, P.; Baruteau, J. Clinical applications for exosomes: are we there yet? *Br. J. Pharmacol.* **2021**, *178* (12), 2375–2392.
- (16) Einabadi, M.; Ai, J.; Kargar, M.; Kafilzadeh, F.; Taghdiri Nooshabadi, V.; Jamali, H. Mesenchymal cell-derived exosomes as novel useful candidates for drug delivery. *Arch. Neurosci.* **2020**, *7*, e98722.
- (17) Yeo, R. W. Y.; Lai, R. C.; Zhang, B.; Tan, S. S.; Yin, Y.; Teh, B. J.; Lim, S. K. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Advanced drug delivery reviews* **2013**, *65* (3), 336–341.
- (18) Song, Y.; Kim, Y.; Ha, S.; Sheller-Miller, S.; Yoo, J.; Choi, C.; Park, C. H. The emerging role of exosomes as novel therapeutics: Biology, technologies, clinical applications, and the next. *American Journal of Reproductive Immunology* **2021**, *85* (2), e13329.
- (19) Ferreira, P. M.; Bozbas, E.; Tannetta, S. D.; Alroqaiba, N.; Zhou, R.; Crawley, J. T. B.; Gibbins, J. M.; Jones, C. I.; Ahnström, J.; Yaqoob, P. Mode of induction of platelet-derived extracellular vesicles is a critical determinant of their phenotype and function. *Sci. Rep.* **2020**, *10* (1), 18061.
- (20) Clark, K.; Zhang, S.; Barthe, S.; Kumar, P.; Pivetti, C.; Kreutzberg, N.; Reed, C.; Wang, Y.; Paxton, Z.; Farmer, D.; et al. Placental mesenchymal stem cell-derived extracellular vesicles promote myelin regeneration in an animal model of multiple sclerosis. *Cells* **2019**, *8* (12), 1497.
- (21) Preußner, C.; Hung, L.-H.; Schneider, T.; Schreiner, S.; Hardt, M.; Moebus, A.; Santoso, S.; Bindereif, A. Selective release of circRNAs in platelet-derived extracellular vesicles. *Journal of extracellular vesicles* **2018**, *7* (1), 1424473.
- (22) Bouchareychas, L.; Duong, P.; Covarrubias, S.; Alsop, E.; Phu, T. A.; Chung, A.; Gomes, M.; Wong, D.; Meechoovet, B.; Capili, A.; et al. Macrophage exosomes resolve atherosclerosis by regulating hematopoiesis and inflammation via MicroRNA cargo. *Cell reports* **2020**, *32* (2), 107881.
- (23) Wei, F.; Li, Z.; Crawford, R.; Xiao, Y.; Zhou, Y. Immunoregulatory role of exosomes derived from differentiating mesenchymal stromal cells on inflammation and osteogenesis. *Journal of tissue engineering and regenerative medicine* **2019**, *13* (11), 1978–1991.
- (24) Cui, X.; Li, J.; Hartanto, Y.; Durham, M.; Tang, J.; Zhang, H.; Hooper, G.; Lim, K.; Woodfield, T. Advances in Extrusion 3D Bioprinting: A Focus on Multicomponent Hydrogel-Based Bioprinting. *Adv. Healthcare Mater.* **2020**, *9* (15), 1901648.
- (25) Sun, Y.; Zhang, B.; Zhai, D.; Wu, C. Three-dimensional printing of bioceramic-induced macrophage exosomes: immunomodulation and osteogenesis/angiogenesis. *NPG Asia Materials* **2021**, *13* (1), 72.
- (26) Liu, A.; Jin, S.; Fu, C.; Cui, S.; Zhang, T.; Zhu, L.; Wang, Y.; Shen, S. G. F.; Jiang, N.; Liu, Y. Macrophage-derived small extracellular vesicles promote biomimetic mineralized collagen-mediated endogenous bone regeneration. *International journal of oral science* **2020**, *12* (1), 33.
- (27) Hu, H.; Dong, L.; Bu, Z.; Shen, Y.; Luo, J.; Zhang, H.; Zhao, S.; Lv, F.; Liu, Z. miR-23a-3p-abundant small extracellular vesicles released from Gelma/nanoclay hydrogel for cartilage regeneration. *Journal of extracellular vesicles* **2020**, *9* (1), 1778883.
- (28) Zhu, D.; Johnson, T. K.; Wang, Y.; Thomas, M.; Huynh, K.; Yang, Q.; Bond, V. C.; Chen, Y. E.; Liu, D. Macrophage M2 polarization induced by exosomes from adipose-derived stem cells contributes to the exosomal proangiogenic effect on mouse ischemic hindlimb. *Stem cell research & therapy* **2020**, *11* (1), 162.
- (29) Wang, J.; Chen, S.; Zhang, W.; Chen, Y.; Bihl, J. C. Exosomes from miRNA-126-modified endothelial progenitor cells alleviate brain injury and promote functional recovery after stroke. *CNS Neuroscience & Therapeutics* **2020**, *26* (12), 1255–1265.
- (30) Yu, T.; Xu, Y.; Ahmad, M. A.; Javed, R.; Hagiwara, H.; Tian, X. Exosomes as a promising therapeutic strategy for peripheral nerve injury. *Current Neuropharmacology* **2021**, *19* (12), 2141–2151.
- (31) Wei, Z.; Fan, B.; Ding, H.; Liu, Y.; Tang, H.; Pan, D.; Shi, J.; Zheng, P.; Shi, H.; Wu, H.; et al. Proteomics analysis of Schwann cell-derived exosomes: a novel therapeutic strategy for central nervous system injury. *Molecular and cellular biochemistry* **2019**, *457* (1–2), 51–59.
- (32) Qing, L.; Chen, H.; Tang, J.; Jia, X. Exosomes and their microRNA cargo: new players in peripheral nerve regeneration. *Neurorehabilitation and neural repair* **2018**, *32* (9), 765–776.
- (33) Simeoli, R.; Montague, K.; Jones, H. R.; Castaldi, L.; Chambers, D.; Kelleher, J. H.; Vacca, V.; Pitcher, T.; Grist, J.; Al-Ahdal, H.; et al. Exosomal cargo including microRNA regulates sensory neuron to macrophage communication after nerve trauma. *Nat. Commun.* **2017**, *8* (1), 1778.
- (34) Wong, F. C.; Ye, L.; Demir, I. E.; Kahlert, C. Schwann cell-derived exosomes: Janus-faced mediators of regeneration and disease. *Glia* **2022**, *70* (1), 20–34.
- (35) Hu, M.; Hong, L.; Liu, C.; Hong, S.; He, S.; Zhou, M.; Huang, G.; Chen, Q. Electrical stimulation enhances neuronal cell activity mediated by Schwann cell derived exosomes. *Sci. Rep.* **2019**, *9* (1), 4206.
- (36) Wu, Z.; Pu, P.; Su, Z.; Zhang, X.; Nie, L.; Chang, Y. Schwann Cell-derived exosomes promote bone regeneration and repair by enhancing the biological activity of porous Ti6Al4V scaffolds. *Biochem. Biophys. Res. Commun.* **2020**, *531* (4), 559–565.
- (37) Newman, E. A.; Abdessalam, S.; Aldrink, J. H.; Austin, M.; Heaton, T. E.; Bruny, J.; Ehrlich, P.; Dasgupta, R.; Baertschiger, R. M.; Lautz, T. B.; Rhee, D. S.; Langham, M. R., Jr.; Malek, M. M.; Meyers, R. L.; Nathan, J. D.; Weil, B. R.; Polites, S.; Madonna, M. B. Update on neuroblastoma. *J. Pediatr. Surg* **2019**, *54* (3), 383–389.
- (38) Neviani, P.; Wise, P. M.; Murtadha, M.; Liu, C. W.; Wu, C. H.; Jong, A. Y.; Seeger, R. C.; Fabbri, M. Natural Killer-Derived Exosomal miR-186 Inhibits Neuroblastoma Growth and Immune Escape Mechanisms. *Cancer Res.* **2019**, *79* (6), 1151–1164.
- (39) Wang, G.; Hu, W.; Chen, H.; Shou, X.; Ye, T.; Xu, Y. Cocktail strategy based on NK cell-derived exosomes and their biomimetic nanoparticles for dual tumor therapy. *Cancers* **2019**, *11* (10), 1560.
- (40) Sharif, S.; Ghahremani, M. H.; Soleimani, M. Differentiation induction and proliferation inhibition by a cell-free approach for delivery of exogenous miRNAs to neuroblastoma cells using mesenchymal stem cells. *Cell Journal (Yakhteh)* **2021**, *22* (4), 556–564.
- (41) Kumar, P.; Becker, J. C.; Gao, K.; Carney, R. P.; Lankford, L.; Keller, B. A.; Herout, K.; Lam, K. S.; Farmer, D. L.; Wang, A. Neuroprotective effect of placenta-derived mesenchymal stromal cells: role of exosomes. *FASEB J.* **2019**, *33* (5), 5836–5849.
- (42) Fonseka, P.; Liem, M.; Ozcitti, C.; Adda, C. G.; Ang, C.-S.; Mathivanan, S. Exosomes from N-Myc amplified neuroblastoma cells induce migration and confer chemoresistance to non-N-Myc amplified cells: Implications of intra-tumour heterogeneity. *Journal of extracellular vesicles* **2019**, *8* (1), 1597614.
- (43) Ma, J.; Xu, M.; Yin, M.; Hong, J.; Chen, H.; Gao, Y.; Xie, C.; Shen, N.; Gu, S.; Mo, X. Exosomal hsa-miR199a-3p promotes proliferation and migration in neuroblastoma. *Frontiers in oncology* **2019**, *9*, 459.
- (44) Chen, W.; Hao, X.; Yang, B.; Zhang, Y.; Sun, L.; Hua, Y.; Yang, L.; Yu, J.; Zhao, J.; Hou, L.; et al. MYCN-amplified neuroblastoma cell-derived exosomal miR-17-5p promotes proliferation and migration of non-MYCN amplified cells. *Molecular Medicine Reports* **2021**, *23* (4), 245.
- (45) Villasante, A.; Godier-Furnemont, A.; Hernandez-Barranco, A.; Le Coq, J.; Boskovic, J.; Peinado, H.; Mora, J.; Samitier, J.; Vunjak-Novakovic, G. Horizontal transfer of the stemness-related markers EZH2 and GLI1 by neuroblastoma-derived extracellular vesicles in stromal cells. *Translational Research* **2021**, *237*, 82–97.
- (46) Morini, M.; Cangelosi, D.; Segalerba, D.; Marimpietri, D.; Raggi, F.; Castellano, A.; Fruci, D.; de Mora, J. F.; Cañete, A.; Yáñez,

- Y.; et al. Exosomal microRNAs from Longitudinal Liquid Biopsies for the Prediction of Response to Induction Chemotherapy in High-Risk Neuroblastoma Patients: A Proof of Concept SIOOPEN Study. *Cancers* **2019**, *11* (10), 1476.
- (47) Mansoor, H.; Ong, H. S.; Riau, A. K.; Stanzel, T. P.; Mehta, J. S.; Yam, G. H.-F. Current trends and future perspective of mesenchymal stem cells and exosomes in corneal diseases. *International journal of molecular sciences* **2019**, *20* (12), 2853.
- (48) Samaeekia, R.; Rabiee, B.; Putra, I.; Shen, X.; Park, Y. J.; Hematti, P.; Eslani, M.; Djililian, A. R. Effect of human corneal mesenchymal stromal cell-derived exosomes on corneal epithelial wound healing. *Investigative ophthalmology & visual science* **2018**, *59* (12), 5194–5200.
- (49) Wang, S.; Hou, Y.; Li, X.; Song, Z.; Sun, B.; Li, X.; Zhang, H. Comparison of exosomes derived from induced pluripotent stem cells and mesenchymal stem cells as therapeutic nanoparticles for treatment of corneal epithelial defects. *Aging (Albany NY)* **2020**, *12* (19), 19546.
- (50) Leszczynska, A.; Kulkarni, M.; Ljubimov, A. V.; Saghizadeh, M. Exosomes from normal and diabetic human corneolimbal keratocytes differentially regulate migration, proliferation and marker expression of limbal epithelial cells. *Sci. Rep.* **2018**, *8* (1), 15173.
- (51) Shen, T.; Zheng, Q.; Luo, H.; Li, X.; Chen, Z.; Song, Z.; Zhou, G.; Hong, C. Exosomal miR-19a from adipose-derived stem cells suppresses differentiation of corneal keratocytes into myofibroblasts. *Aging (Albany NY)* **2020**, *12* (5), 4093.
- (52) Shojaati, G.; Khandaker, I.; Funderburgh, M. L.; Mann, M. M.; Basu, R.; Stolz, D. B.; Geary, M. L.; Dos Santos, A.; Deng, S. X.; Funderburgh, J. L. Mesenchymal stem cells reduce corneal fibrosis and inflammation via extracellular vesicle-mediated delivery of miRNA. *Stem cells translational medicine* **2019**, *8* (11), 1192–1201.
- (53) Hu, X.; Zhong, Y.; Kong, Y.; Chen, Y.; Feng, J.; Zheng, J. Lineage-specific exosomes promote the odontogenic differentiation of human dental pulp stem cells (DPSCs) through TGF $\beta$ 1/smads signaling pathway via transfer of microRNAs. *Stem cell research & therapy* **2019**, *10* (1), 170.
- (54) Venugopal, C.; K, S.; Rai, K. S.; Pinnelli, V. B.; Kutty, B. M.; Dhanushkodi, A. Neuroprotection by human dental pulp mesenchymal stem cells: from billions to nano. *Current gene therapy* **2018**, *18* (5), 307–323.
- (55) Wang, H. S.; Yang, F. H.; Wang, Y. J.; Pei, F.; Chen, Z.; Zhang, L. Odontoblastic exosomes attenuate apoptosis in neighboring cells. *Journal of dental research* **2019**, *98* (11), 1271–1278.
- (56) Lin, T.; Wu, N.; Wang, L.; Zhang, R.; Pan, R.; Chen, Y.-F. Inhibition of chondrocyte apoptosis in a rat model of osteoarthritis by exosomes derived from miR-140-5p-overexpressing human dental pulp stem cells. *Int. J. Mol. Med.* **2020**, *47* (3), 7.
- (57) Jarmalavičiūtė, A.; Tunaitis, V.; Pivoraite, U.; Venalis, A.; Pivoriūnas, A. Exosomes from dental pulp stem cells rescue human dopaminergic neurons from 6-hydroxy-dopamine-induced apoptosis. *Cytotherapy* **2015**, *17* (7), 932–939.
- (58) Shen, Z.; Kuang, S.; Zhang, Y.; Yang, M.; Qin, W.; Shi, X.; Lin, Z. Chitosan hydrogel incorporated with dental pulp stem cell-derived exosomes alleviates periodontitis in mice via a macrophage-dependent mechanism. *Bioactive materials* **2020**, *5* (4), 1113–1126.
- (59) Li, S.; Luo, L.; He, Y.; Li, R.; Xiang, Y.; Xing, Z.; Li, Y.; Albashari, A. A.; Liao, X.; Zhang, K.; et al. Dental pulp stem cell-derived exosomes alleviate cerebral ischaemia-reperfusion injury through suppressing inflammatory response. *Cell proliferation* **2021**, *54* (8), e13093.
- (60) Ji, L.; Bao, L.; Gu, Z.; Zhou, Q.; Liang, Y.; Zheng, Y.; Xu, Y.; Zhang, X.; Feng, X. Comparison of immunomodulatory properties of exosomes derived from bone marrow mesenchymal stem cells and dental pulp stem cells. *Immunologic research* **2019**, *67* (4), 432–442.
- (61) Li, J.; Ju, Y.; Liu, S.; Fu, Y.; Zhao, S. Exosomes derived from lipopolysaccharide-preconditioned human dental pulp stem cells regulate Schwann cell migration and differentiation. *Connective tissue research* **2021**, *62* (3), 277–286.
- (62) Ivica, A.; Ghayor, C.; Zehnder, M.; Valdec, S.; Weber, F. E. Pulp-derived exosomes in a fibrin-based regenerative root filling material. *Journal of clinical medicine* **2020**, *9* (2), 491.
- (63) Shimizu, Y.; Takeda-Kawaguchi, T.; Kuroda, I.; Hotta, Y.; Kawasaki, H.; Hariyama, T.; Shibata, T.; Akao, Y.; Kunisada, T.; Tatsumi, J.; et al. Exosomes from dental pulp cells attenuate bone loss in mouse experimental periodontitis. *Journal of Periodontal Research* **2022**, *57*, 162.
- (64) Swanson, W. B.; Zhang, Z.; Xiu, K.; Gong, T.; Eberle, M.; Wang, Z.; Ma, P. X. Scaffolds with controlled release of pro-mineralization exosomes to promote craniofacial bone healing without cell transplantation. *Acta Biomaterialia* **2020**, *118*, 215–232.
- (65) Chiricosta, L.; Silvestro, S.; Gugliandolo, A.; Marconi, G. D.; Pizzicannella, J.; Bramanti, P.; Trubiani, O.; Mazzon, E. Extracellular Vesicles of Human Periodontal Ligament Stem Cells Contain MicroRNAs Associated to Proto-Oncogenes: Implications in Cytokinesis. *Frontiers in Genetics* **2020**, *11*, 582.
- (66) Liu, T.; Hu, W.; Zou, X.; Xu, J.; He, S.; Chang, L.; Li, X.; Yin, Y.; Tian, M.; Li, Z.; et al. Human periodontal ligament stem cell-derived exosomes promote bone regeneration by altering microRNA profiles. *Stem Cells International* **2020**, *2020*, 8852307.
- (67) Čebatariūnienė, A.; Kriaučiūnaitė, K.; Prunskaitė, J.; Tunaitis, V.; Pivoriūnas, A. Extracellular vesicles suppress basal and lipopolysaccharide-induced NF $\kappa$ B activity in human periodontal ligament stem cells. *Stem cells and development* **2019**, *28* (15), 1037–1049.
- (68) Tatullo, M.; Codispoti, B.; Spagnuolo, G.; Zavan, B. Human periapical cyst-derived stem cells can be a smart “lab-on-a-cell” to investigate neurodegenerative diseases and the related alteration of the exosomes’ content. *Brain sciences* **2019**, *9* (12), 358.
- (69) Pizzicannella, J.; Gugliandolo, A.; Orsini, T.; Fontana, A.; Ventrella, A.; Mazzon, E.; Bramanti, P.; Diomedea, F.; Trubiani, O. Engineered extracellular vesicles from human periodontal-ligament stem cells increase VEGF/VEGFR2 expression during bone regeneration. *Frontiers in physiology* **2019**, *10*, 512.
- (70) Zhang, J.; Buller, B. A.; Zhang, Z. G.; Zhang, Y.; Lu, M.; Rosene, D. L.; Medalla, M.; Moore, T. L.; Chopp, M. Exosomes derived from bone marrow mesenchymal stromal cells promote remyelination and reduce neuroinflammation in the demyelinating central nervous system. *Exp. Neurol.* **2022**, *347*, 113895.
- (71) El-Derany, M. O.; Noureldein, M. H. Bone marrow mesenchymal stem cells and their derived exosomes resolve doxorubicin-induced chemobrain: critical role of their miRNA cargo. *Stem Cell Research & Therapy* **2021**, *12* (1), 322.
- (72) Xiao, Y.; Geng, F.; Wang, G.; Li, X.; Zhu, J.; Zhu, W. Bone marrow-derived mesenchymal stem cells-derived exosomes prevent oligodendrocyte apoptosis through exosomal miR-134 by targeting caspase-8. *Journal of cellular biochemistry* **2019**, *120* (2), 2109–2118.
- (73) Li, C.; Li, X.; Zhao, B.; Wang, C. Exosomes derived from miR-544-modified mesenchymal stem cells promote recovery after spinal cord injury. *Archives of physiology and biochemistry* **2020**, *126* (4), 369–375.
- (74) Jia, Y.; Yang, J.; Lu, T.; Pu, X.; Chen, Q.; Ji, L.; Luo, C. Repair of spinal cord injury in rats via exosomes from bone mesenchymal stem cells requires sonic hedgehog. *Regen Ther* **2021**, *18*, 309–315.
- (75) Zhou, Y.; Wen, L. L.; Li, Y. F.; Wu, K. M.; Duan, R. R.; Yao, Y. B.; Jing, L. J.; Gong, Z.; Teng, J. F.; Jia, Y. J. Exosomes derived from bone marrow mesenchymal stem cells protect the injured spinal cord by inhibiting pericyte pyroptosis. *Neural Regen Res.* **2022**, *17* (1), 194–202.
- (76) Yuan, F. Y.; Zhang, M. X.; Shi, Y. H.; Li, M. H.; Ou, J. Y.; Bai, W. F.; Zhang, M. S. Bone marrow stromal cells-derived exosomes target DAB2IP to induce microglial cell autophagy, a new strategy for neural stem cell transplantation in brain injury. *Exp Ther Med.* **2020**, *20* (3), 2752–2764.
- (77) Zhang, J.; Rong, Y.; Luo, C.; Cui, W. Bone marrow mesenchymal stem cell-derived exosomes prevent osteoarthritis by regulating synovial macrophage polarization. *Aging (Albany NY)* **2020**, *12* (24), 25138–25152.

- (78) Mendes-Pinheiro, B.; Anjo, S. I.; Manadas, B.; Da Silva, J. D.; Marote, A.; Behie, L. A.; Teixeira, F. G.; Salgado, A. J. Bone Marrow Mesenchymal Stem Cells' Secretome Exerts Neuroprotective Effects in a Parkinson's Disease Rat Model. *Front Bioeng Biotechnol* **2019**, *7*, 294.
- (79) Su, Y.; Liu, Y.; Ma, C.; Guan, C.; Ma, X.; Meng, S. Mesenchymal stem cell-originated exosomal lncRNA HAND2-AS1 impairs rheumatoid arthritis fibroblast-like synoviocyte activation through miR-143-3p/TNFAIP3/NF- $\kappa$ B pathway. *J. Orthop Surg Res.* **2021**, *16* (1), 116.
- (80) Takeuchi, R.; Katagiri, W.; Endo, S.; Kobayashi, T. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. *PLoS One* **2019**, *14* (11), e0225472.
- (81) Guo, S.; Gu, J.; Ma, J.; Xu, R.; Wu, Q.; Meng, L.; Liu, H.; Li, L.; Xu, Y. GATA4-driven miR-206-3p signatures control orofacial bone development by regulating osteogenic and osteoclastic activity. *Theranostics* **2021**, *11* (17), 8379–8395.
- (82) Wei, R.; Zhang, L.; Hu, W.; Shang, X.; He, Y.; Zhang, W. Zeb2/Axin2-Enriched BMSC-Derived Exosomes Promote Post-Stroke Functional Recovery by Enhancing Neurogenesis and Neural Plasticity. *Journal of Molecular Neuroscience* **2022**, *72* (1), 69–81.
- (83) Riazifar, M.; Mohammadi, M. R.; Pone, E. J.; Yeri, A.; Lasser, C.; Segaliny, A. I.; McIntyre, L. L.; Shelke, G. V.; Hutchins, E.; Hamamoto, A.; et al. Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders. *ACS Nano* **2019**, *13* (6), 6670–6688.
- (84) Vilaça-Faria, H.; Salgado, A. J.; Teixeira, F. G. Mesenchymal stem cells-derived exosomes: a new possible therapeutic strategy for Parkinson's disease? *Cells* **2019**, *8* (2), 118.
- (85) Shiue, S.-J.; Rau, R.-H.; Shiue, H.-S.; Hung, Y.-W.; Li, Z.-X.; Yang, K. D.; Cheng, J.-K. Mesenchymal stem cell exosomes as a cell-free therapy for nerve injury-induced pain in rats. *Pain* **2019**, *160* (1), 210–223.
- (86) Lee, M.; Liu, T.; Im, W.; Kim, M. Exosomes from adipose-derived stem cells ameliorate phenotype of Huntington's disease in vitro model. *European Journal of Neuroscience* **2016**, *44* (4), 2114–2119.
- (87) Lee, M.; Ban, J.-J.; Yang, S.; Im, W.; Kim, M. The exosome of adipose-derived stem cells reduces  $\beta$ -amyloid pathology and apoptosis of neuronal cells derived from the transgenic mouse model of Alzheimer's disease. *Brain research* **2018**, *1691*, 87–93.
- (88) Lee, M.; Ban, J.-J.; Kim, K. Y.; Jeon, G. S.; Im, W.; Sung, J.-J.; Kim, M. Adipose-derived stem cell exosomes alleviate pathology of amyotrophic lateral sclerosis in vitro. *Biochemical and biophysical research communications* **2016**, *479* (3), 434–439.
- (89) Wang, L.; Chopp, M.; Szalad, A.; Lu, X.; Zhang, Y.; Wang, X.; Cepparulo, P.; Lu, M.; Li, C.; Zhang, Z. G. Exosomes derived from Schwann cells ameliorate peripheral neuropathy in type 2 diabetic mice. *Diabetes* **2020**, *69* (4), 749–759.
- (90) Ching, R. C.; Wiberg, M.; Kingham, P. J. Schwann cell-like differentiated adipose stem cells promote neurite outgrowth via secreted exosomes and RNA transfer. *Stem cell research & therapy* **2018**, *9* (1), 266.
- (91) Frühbeis, C.; Kuo-Elsner, W. P.; Müller, C.; Barth, K.; Peris, L.; Tenzer, S.; Möbius, W.; Werner, H. B.; Nave, K.-A.; Fröhlich, D.; et al. Oligodendrocytes support axonal transport and maintenance via exosome secretion. *PLoS biology* **2020**, *18* (12), e3000621.
- (92) Yu, Z.; Shi, M.; Stewart, T.; Fernagut, P.-O.; Huang, Y.; Tian, C.; Dehay, B.; Atik, A.; Yang, D.; De Giorgi, F.; et al. Reduced oligodendrocyte exosome secretion in multiple system atrophy involves SNARE dysfunction. *Brain* **2020**, *143* (6), 1780–1797.
- (93) Guo, M.; Wang, J.; Zhao, Y.; Feng, Y.; Han, S.; Dong, Q.; Cui, M.; Tieu, K. Microglial exosomes facilitate  $\alpha$ -synuclein transmission in Parkinson's disease. *Brain* **2020**, *143* (5), 1476–1497.
- (94) Gao, Z.-S.; Zhang, C.-J.; Xia, N.; Tian, H.; Li, D.-Y.; Lin, J.-Q.; Mei, X.-F.; Wu, C. Berberine-loaded M2 macrophage-derived exosomes for spinal cord injury therapy. *Acta Biomaterialia* **2021**, *126*, 211–223.
- (95) Du, T.; Yang, C.-L.; Ge, M.-R.; Liu, Y.; Zhang, P.; Li, H.; Li, X.-L.; Li, T.; Liu, Y.-D.; Dou, Y.-C.; et al. M1 macrophage derived exosomes aggravate experimental autoimmune neuritis via modulating Th1 response. *Frontiers in immunology* **2020**, *11*, 1603.
- (96) Zheng, Y.; He, R.; Wang, P.; Shi, Y.; Zhao, L.; Liang, J. Exosomes from LPS-stimulated macrophages induce neuroprotection and functional improvement after ischemic stroke by modulating microglial polarization. *Biomaterials science* **2019**, *7* (5), 2037–2049.
- (97) Xiao, B.; Chai, Y.; Lv, S.; Ye, M.; Wu, M.; Xie, L.; Fan, Y.; Zhu, X.; Gao, Z. Endothelial cell-derived exosomes protect SH-SY5Y nerve cells against ischemia/reperfusion injury. *International journal of molecular medicine* **2017**, *40* (4), 1201–1209.
- (98) Jiang, M.; Wang, H.; Jin, M.; Yang, X.; Ji, H.; Jiang, Y.; Zhang, H.; Wu, F.; Wu, G.; Lai, X.; et al. Exosomes from MiR-30d-5p-ADSCs reverse acute ischemic stroke-induced, autophagy-mediated brain injury by promoting M2 microglial/macrophage polarization. *Cellular Physiology and Biochemistry* **2018**, *47* (2), 864–878.
- (99) Ling, X.; Zhang, G.; Xia, Y.; Zhu, Q.; Zhang, J.; Li, Q.; Niu, X.; Hu, G.; Yang, Y.; Wang, Y.; et al. Exosomes from human urine-derived stem cells enhanced neurogenesis via miR-26a/HDAC6 axis after ischaemic stroke. *Journal of cellular and molecular medicine* **2020**, *24* (1), 640–654.
- (100) Williams, A. M.; Higgins, G. A.; Bhatti, U. F.; Biesterveld, B. E.; Dekker, S. E.; Kathawate, R. G.; Tian, Y.; Wu, Z.; Kemp, M. T.; Wakam, G. K.; et al. Early treatment with exosomes following traumatic brain injury and hemorrhagic shock in a swine model promotes transcriptional changes associated with neuroprotection. *Journal of Trauma and Acute Care Surgery* **2020**, *89* (3), 536–543.
- (101) Zhang, Z.; Zou, X.; Zhang, R.; Xie, Y.; Feng, Z.; Li, F.; Han, J.; Sun, H.; Ouyang, Q.; Hua, S.; et al. Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation via suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke. *Aging (Albany NY)* **2021**, *13* (2), 3060.
- (102) Nalamolu, K. R.; Venkatesh, I.; Mohandass, A.; Klopfenstein, J. D.; Pinson, D. M.; Wang, D. Z.; Kunamneni, A.; Veeravalli, K. K. Exosomes secreted by the cocultures of normal and oxygen-glucose-deprived stem cells improve post-stroke outcome. *Neuromolecular medicine* **2019**, *21* (4), 529–539.
- (103) Jiang, Y.; He, R.; Shi, Y.; Liang, J.; Zhao, L. Plasma exosomes protect against cerebral ischemia/reperfusion injury via exosomal HSP70 mediated suppression of ROS. *Life sciences* **2020**, *256*, 117987.
- (104) Guo, L.; Pan, J.; Li, F.; Zhao, L.; Shi, Y. A novel brain targeted plasma exosomes enhance the neuroprotective efficacy of edaravone in ischemic stroke. *IET Nanobiotechnology* **2021**, *15* (1), 107–116.
- (105) Tian, T.; Zhang, H.-X.; He, C.-P.; Fan, S.; Zhu, Y.-L.; Qi, C.; Huang, N.-P.; Xiao, Z.-D.; Lu, Z.-H.; Tannous, B. A.; et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* **2018**, *150*, 137–149.
- (106) Baharloo, H.; Nouraei, Z.; Azimi, M.; Moghadasi, A. N.; Tavassolifar, M. J.; Moradi, B.; Sahraian, M. A.; Izad, M. Umbilical cord mesenchymal stem cells as well as their released exosomes suppress proliferation of activated PBMCs in multiple sclerosis. *Scandinavian Journal of Immunology* **2021**, *93*, e13013.
- (107) Azimi, M.; Ghabaee, M.; Moghadasi, A. N.; Izad, M. Altered expression of miR-326 in T cell-derived exosomes of patients with relapsing-remitting multiple sclerosis. *Iranian Journal of Allergy, Asthma and Immunology* **2019**, DOI: 10.18502/ijaai.v18i1.636.
- (108) Kimura, K.; Hohjoh, H.; Fukuoka, M.; Sato, W.; Oki, S.; Tomi, C.; Yamaguchi, H.; Kondo, T.; Takahashi, R.; Yamamura, T. Circulating exosomes suppress the induction of regulatory T cells via let-7i in multiple sclerosis. *Nat. Commun.* **2018**, *9* (1), 17.
- (109) Benjamins, J. A.; Nedelkoska, L.; Touil, H.; Stemmer, P. M.; Caruthers, N. J.; Jena, B. P.; Naik, A. R.; Bar-Or, A.; Lisak, R. P. Exosome-enriched fractions from MS B cells induce oligodendrocyte death. *Neurology-Neuroimmunology Neuroinflammation* **2019**, *6* (3), e550.
- (110) Li, Z.; Liu, F.; He, X.; Yang, X.; Shan, F.; Feng, J. Exosomes derived from mesenchymal stem cells attenuate inflammation and

demyelination of the central nervous system in EAE rats by regulating the polarization of microglia. *International immunopharmacology* **2019**, *67*, 268–280.

(111) Kojima, R.; Bojar, D.; Rizzi, G.; Hamri, G. C.-E.; El-Baba, M. D.; Saxena, P.; Auslander, S.; Tan, K. R.; Fussenegger, M. Designer exosomes produced by implanted cells intracerebrally deliver therapeutic cargo for Parkinson's disease treatment. *Nat. Commun.* **2018**, *9* (1), 1305.

(112) Chen, H.-X.; Liang, F.-C.; Gu, P.; Xu, B.-L.; Xu, H.-J.; Wang, W.-T.; Hou, J.-Y.; Xie, D.-X.; Chai, X.-Q.; An, S.-J. Exosomes derived from mesenchymal stem cells repair a Parkinson's disease model by inducing autophagy. *Cell death & disease* **2020**, *11* (4), 288.

(113) Qu, M.; Lin, Q.; Huang, L.; Fu, Y.; Wang, L.; He, S.; Fu, Y.; Yang, S.; Zhang, Z.; Zhang, L.; et al. Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease. *Journal of controlled release* **2018**, *287*, 156–166.

(114) Liu, L.; Li, Y.; Peng, H.; Liu, R.; Ji, W.; Shi, Z.; Shen, J.; Ma, G.; Zhang, X. Targeted exosome coating gene-chem nanocomplex as "nanoscavenger" for clearing  $\alpha$ -synuclein and immune activation of Parkinson's disease. *Science advances* **2020**, *6* (50), eaba3967.

(115) Ren, X.; Zhao, Y.; Xue, F.; Zheng, Y.; Huang, H.; Wang, W.; Chang, Y.; Yang, H.; Zhang, J. Exosomal DNA aptamer targeting  $\alpha$ -synuclein aggregates reduced neuropathological deficits in a mouse Parkinson's disease model. *Molecular Therapy-Nucleic Acids* **2019**, *17*, 726–740.

(116) Sun, T.; Ding, Z.-X.; Luo, X.; Liu, Q.-S.; Cheng, Y. Blood exosomes have neuroprotective effects in a mouse model of Parkinson's disease. *Oxidative medicine and cellular longevity* **2020**, *2020*, 1.

(117) Li, Q.; Wang, Z.; Xing, H.; Wang, Y.; Guo, Y. Exosomes derived from miR-188-3p-modified adipose-derived mesenchymal stem cells protect Parkinson's disease. *Molecular Therapy-Nucleic Acids* **2021**, *23*, 1334–1344.

(118) Wang, H.; Sui, H.; Zheng, Y.; Jiang, Y.; Shi, Y.; Liang, J.; Zhao, L. Curcumin-primed exosomes potently ameliorate cognitive function in AD mice by inhibiting hyperphosphorylation of the Tau protein through the AKT/GSK-3 $\beta$  pathway. *Nanoscale* **2019**, *11* (15), 7481–7496.

(119) Qi, Y.; Guo, L.; Jiang, Y.; Shi, Y.; Sui, H.; Zhao, L. Brain delivery of quercetin-loaded exosomes improved cognitive function in AD mice by inhibiting phosphorylated tau-mediated neurofibrillary tangles. *Drug Delivery* **2020**, *27* (1), 745–755.

(120) Chen, Y.-A.; Lu, C.-H.; Ke, C.-C.; Chiu, S.-J.; Jeng, F.-S.; Chang, C.-W.; Yang, B.-H.; Liu, R.-S. Mesenchymal stem cell-derived exosomes ameliorate Alzheimer's disease pathology and improve cognitive deficits. *Biomedicines* **2021**, *9* (6), 594.

(121) Wang, X.; Yang, G. Bone marrow mesenchymal stem cells-derived exosomes reduce A $\beta$  deposition and improve cognitive function recovery in mice with Alzheimer's disease by activating sphingosine kinase/sphingosine-1-phosphate signaling pathway. *Cell Biol. Int.* **2021**, *45* (4), 775–784.

(122) Nakano, M.; Kubota, K.; Kobayashi, E.; Chikenji, T. S.; Saito, Y.; Konari, N.; Fujimiya, M. Bone marrow-derived mesenchymal stem cells improve cognitive impairment in an Alzheimer's disease model by increasing the expression of microRNA-146a in hippocampus. *Sci. Rep.* **2020**, *10* (1), 10772.

(123) Jahangard, Y.; Monfared, H.; Moradi, A.; Zare, M.; Mirnajafi-Zadeh, J.; Mowla, S. J. Therapeutic effects of transplanted exosomes containing miR-29b to a rat model of Alzheimer's disease. *Frontiers in Neuroscience* **2020**, *14*, 564.

(124) Zhai, L.; Shen, H.; Sheng, Y.; Guan, Q. ADMSC Exo-MicroRNA-22 improve neurological function and neuroinflammation in mice with Alzheimer's disease. *Journal of Cellular and Molecular Medicine* **2021**, *25* (15), 7513–7523.

(125) Cui, G.-h.; Guo, H.-d.; Li, H.; Zhai, Y.; Gong, Z.-b.; Wu, J.; Liu, J.-s.; Dong, Y.-r.; Hou, S.-x.; Liu, J.-r. RVG-modified exosomes derived from mesenchymal stem cells rescue memory deficits by regulating inflammatory responses in a mouse model of Alzheimer's disease. *Immunity & Ageing* **2019**, *16* (1), 10.

(126) Pan, J.; He, R.; Huo, Q.; Shi, Y.; Zhao, L. Brain Microvascular Endothelial Cell Derived Exosomes Potently Ameliorate Cognitive Dysfunction by Enhancing the Clearance of A $\beta$  Through Up-Regulation of P-gp in Mouse Model of AD. *Neurochemical research* **2020**, *45* (9), 2161–2172.