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# Extracellular Vesicles from Stem and Progenitor Cells for Cell-Free Regenerative Therapy

Nazmul Haque<sup>\*a</sup>, Darius Widera<sup>b</sup>, Vijayendran Govindasamy<sup>c</sup>, Pratiwi Soesilawati<sup>d</sup>, Noor Hayaty Abu Kasim<sup>\*de</sup>

<sup>a</sup>*Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, MAHSA University, Selangor, Malaysia.*

<sup>b</sup>*Stem Cell Biology and Regenerative Medicine Group, School of Pharmacy, University of Reading, Reading, UK*

<sup>c</sup>*Cryocord Malaysia Sdn Bhd, Cyberjaya, Selangor, Malaysia*

<sup>d</sup>*Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*

<sup>e</sup>*Faculty of Dentistry, Universiti Kebaangsan Malaysia, Kuala Lumpur, Malaysia.*

**Abstract:** Cell-based regenerative therapies involving stem or progenitor cells are considered as possible therapeutic modalities to treat non-communicable and degenerative diseases. Recently, regenerative outcomes of cell-based therapies have been linked to paracrine factors and extracellular vesicles [EVs] released by the transplanted cells rather than the transplanted cells themselves. EVs contain a cargo that includes microRNAs [miRNAs], mRNAs, as well as proteins. Their role in mediating intercellular communication has been acknowledged in several studies. However, the regenerative potential of the miRNAs, mRNAs, and proteins that are present in EVs is a matter of ongoing scientific debate. In this review, we discuss EVs as an alternative to stem cell-based therapy to treat some of the non-communicable and degenerative diseases. Moreover, we also propose that pre-treatment of the cells could help to produce EVs enriched with particular miRNAs, mRNAs, and/or proteins that could support the successful regeneration of a targeted organ.

**Keywords:** exosomes; mesenchymal stem cells; microvesicles; miRNA; mRNA; paracrine factor.

## 1. INTRODUCTION

In the last four decades, the average human life expectancy at birth has increased by about 10 years [1-3]. However, simultaneously, a 9 years gap between the total life expectancy and healthy life expectancy has also been reported [1]. This gap between life expectancy and healthy life expectancy indicates that a large number of people around the world are living with medical conditions. Non-communicable and age-related degenerative diseases are considered the major contributors to these morbid conditions [1-3]. There are several symptomatic treatments available to treat non-communicable or degenerative diseases. However, till now there is no effective therapeutic modality that could help to regenerate affected organs. Often, organ transplantation is the only option, whereas scarcity in donor organs and immunologically mediated graft loss following transplantation are the major drawbacks [4, 5].

Several *in vitro*, *in vivo*, and clinical studies have been conducted with a large variety of stem cell types including induced pluripotent stem cells [iPSCs], mesenchymal stem cells [MSCs], hematopoietic stem cells [HSCs], blast cells, and progenitor cells. Their promising regenerative outcomes are considered as hope to resolve the issue of organ donor scarcity in the near future [6-15].

\*Address correspondence to this author at the Noor Hayaty Abu Kasim, Faculty of Dentistry, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, MALAYSIA. E-mail: [nhayat@ukm.edu.my](mailto:nhayat@ukm.edu.my)

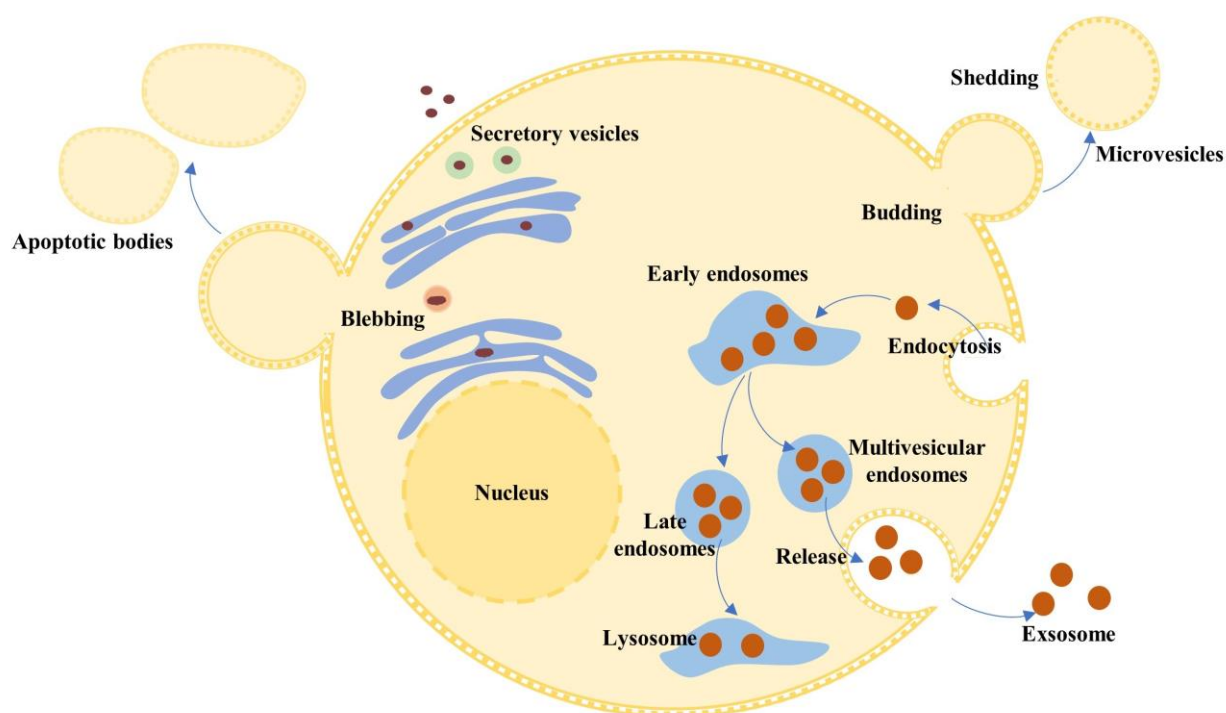
Nazmul Haque, Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, MAHSA University, 42610 Selangor, MALAYSIA.

E-mail: [tanna.bge@gmail.com](mailto:tanna.bge@gmail.com); [nazmul@mahsa.edu.my](mailto:nazmul@mahsa.edu.my)

However, several studies have suggested that the regenerative outcomes of cell therapy, especially of MSCs based regenerative therapy, are largely mediated by paracrine factors rather than by engraftment and differentiation of the transplanted cells [16-18]. This emphasizes the regenerative potential of stem cell-derived paracrine factors and extracellular vesicles [EVs]. This opened a new opportunity in the field of regenerative medicine and led to multiple studies exploring the potential of stem cell secretome and EVs as cell-free regenerative therapeutics.

Release of EVs by reticulocytes into the extracellular space was observed and reported in 1980s [19, 20]. Until 1996, before exploring the role of EVs in stimulating an adaptive immune response [21], they were considered as cell debris without biological relevance [22]. After that discovery, EVs received considerable attention due to their ability to mediate intercellular communication.

Based on their biogenesis, EVs can be divided into exosomes, microvesicles, and apoptotic bodies [Figure 1] [22]. Exosomes are released by cells upon fusion of multivesicular endosomes with the plasma membrane [23] and range in size from 30-120 nm in diameter [24]. Microvesicles originate from the cell surface by direct outward budding and fission of the plasma membrane [24]. They are more heterogeneous compared to exosomes and range from 50-1000 nm in diameter [25]. Among different types of EVs, apoptotic bodies that are generated through a process known as apoptotic cell disassembly, represent the largest EVs with an average diameter of 1-5  $\mu\text{m}$  [26].



**Figure 1** Different types of extracellular vesicles (EVs) and their biogenesis.

EVs contain a cargo that includes microRNAs [miRNAs], mRNAs, and proteins which play a vital role in maintaining intercellular communication [26, 27]. In recent years, the regenerative potential of EVs [especially exosomes and microvesicles] has also received considerable attention. In this review, the regenerative potential and future prospects of EVs as cell-free therapeutics will be discussed.

## 2. POTENTIAL USES OF EVS IN REGENERATIVE THERAPY

Cell-cell communication through EVs is a bi-directional process [28]. Usually, the biogenesis and the content of EVs are closely linked to the cross-talk between cells and their niche. Cells of origin and the physiologic or pathologic condition of the microenvironment regulate the content of the EVs and subsequently their biological functions [29]. Following secretion, EVs carry signaling molecules [miRNAs, mRNA, and proteins] to adjacent or distant cells and play an important role in maintaining cell-cell communication [30]. The EVs originating from a particular cell type can regulate the phenotype and fate of the recipient cells by transferring cell surface receptors, genetic material, and/or transcriptional regulators [31, 32]. The regenerative potential of EVs from different cell types has been studied *in vitro* and *in vivo*. In the following, we will discuss the potential of EVs in treating selected pathological conditions including cardiac, liver, and neuronal degeneration [Figure 2].

### 2.1. Cardiac Regeneration

In the last few decades, myocardial infarction has been recognized as one of the major causes of mortality and morbidity worldwide. It also has a significant impact on the socio-economic status and quality of life of the survivors [33]. Cell-cell communication or cross-talk between different cell types including cardiac progenitor cells, cardiomyocytes, endothelial cells, and fibroblasts play vital roles in cardiac regeneration and protection of the ischemic myocardium. To maintain homeostasis within heart tissue, cardiomyocytes send messages to cardiac progenitor cells, endothelial cells, and fibroblasts while receiving reciprocal signals from these cell types [34].

Endothelial progenitor cells [EPCs] and MSCs have the potential to protect the ischemic myocardium [35]. Recently, it has been established that EVs are the key component of cell-cell communication and the cardiac regenerative outcomes of the cell-based therapy are due to cell-secreted paracrine factors embedded within EVs [33, 36, 37]. In this context, the protective effects of hiPSC- and cardiomyocyte-derived EVs on ischemic myocardium have been reported [34, 38]. However, the molecular mechanisms behind the EV-mediated myocardial protection are still illusive.

Previously, it has been hypothesized that proteomic deficiencies during ischemia-reperfusion injury may be linked to the loss of adenosine triphosphate [ATP]/nicotinamide adenine dinucleotide hydrogen [NADH], resulting in increased oxidative stress and cell death [39, 40]. Increased ATP and NADH levels, decreased oxidative stress, increased phosphorylated protein kinase B [p-Akt] phosphorylated glycogen synthase kinase-3 [p-GSK-3] beta, and reduced C-Jun N-terminal kinase [p-c-JNK] within one hour after reperfusion in ischemic mice hearts treated

with MSC-derived EVs [MSC-EVs] have been reported. Furthermore, 24 h post-reperfusion, a significant reduction in local and systemic inflammation, and a 45% reduction in infarct size were observed in MSC-EVs treated animals. Higher contractility and relaxation have also been observed during a 28 days follow-up. However, the effect was confined to the intact EVs only [40]. In another study, it has been found that cluster of differentiation [CD] 81, CD9, and Alix markers containing purified EVs reduced infarct size in the mouse of myocardial ischemia-reperfusion injury model [37].

Several *in vitro* and *in vivo* studies have linked the presence of miRNAs in EVs to the protection of cardiomyocytes, and induction of angiogenesis [34, 41]. In an *in vivo* study, intravenously injected apoptotic human umbilical vein endothelial cell [HUVEC]-EVs induced a reduction of inflammatory atherosclerotic plaques [42]. This regenerative outcome was further linked to the presence of miR-126 in the EV fraction that induced the cardiac protection by increasing the expression of stromal cell-derived factor 1 [SDF-1] in recipient cells [42]. *In vitro* protection of cardiac progenitor cells [CPCs] under ischemic condition by miR-126 and miR-210 enriched EVs have been acknowledged in another study [43]. Similar observations have been reported for the EVs containing miR-210, miR-132, and miR-146a released by cardiosphere-derived cells and CPCs [41, 44]. Recently, multilayered composite membranes of biomaterials have been reported to increase the secretion of EVs from EPCs in which a higher amount of miR-126 [45]. The results of this study suggest that the culture of stem cells on the multilayered composite membrane of biomaterials could help to produce EVs with higher angiogenic potential.

In addition to miRNAs, EV cargo also contains multiple proteins. Under physiological conditions, [SDF-1]/ C-X-C motif chemokine receptor [CXCR4] signaling has been reported to be involved in enhanced endothelial sprouting from human embryoid bodies [46]. This result signifies the role of CXCR4 in regulating initial vessel formation which is highly important for cardiac regeneration. In another study, EVs from MSCs transduced with lentiviral CXCR4 have been found to promote functional cardiac restoration [increased angiogenesis, reduced infarct size, and improved cardiac remodeling] *in vivo* via upregulating the expression of insulin-like growth factor 1 alpha [IGF-1 $\alpha$ ] alpha, pAkt, vascular endothelial growth factor [VEGF], as well as by downregulating the levels of active caspase 3 in cardiomyocytes [47]. Further studies are needed to reveal and specify the role of EVs from different sources or the components of EVs in cardiac regeneration.

### 2.2. Liver Regeneration

Hepatic fibrosis is a process that results from imbalanced accumulation and degradation of the extracellular matrix [48]. It is triggered by chronic liver injuries. If untreated, it may cause life-threatening liver cirrhosis and hepatocellular carcinomas. Chronic inflammation of hepatic cells is frequently caused by viral infections, alcoholic liver disease, non-alcoholic fatty liver disease, and autoimmune attack. Liver cirrhosis an end-stage sequela of fibrosis is one of the major causes of morbidity and mortality worldwide. At present, there is no pharmaceutical option for treating hepatic fibrosis, and liver transplantation is considered the only curative treatment for end-stage cirrhosis [5]. However, scarcity in the donor organs even in the developed countries as well

as low survival rates of grafts following liver transplantation are the major limitations of this treatment modality. These obstacles intensified the need of new medical therapies to attenuate or reverse hepatic fibrosis.

Several *in vitro* and *in vivo* studies have demonstrated the hepatic regenerative potential of EVs from different cell types. *In vitro* inhibition of growth and survival of HepG2 hepatoma and primary hepatocellular carcinoma cells in the presence of human adult liver stem cells [HLSC]-EVs have been reported earlier [49]. Furthermore, intra-tumor administration of HLSC-EVs have shown regression of ectopic tumors in severe combined immunodeficiency [SCID] mice [49]. These hepatoprotective effects of HLSC-EVs were linked to a horizontal transfer of miRNAs from EVs to the target cells [49]. In another study, EVs from bone marrow [BM]-MSCs also induced apoptosis of HepG2 and Kaposi's cells as well as necrosis in Skov-3 *in vitro* in addition to an inhibition of progression of established tumors *in vivo* [50].

EVs from different cell sources have also been shown to have the potential to minimize ischemia-reperfusion and toxin-induced hepatic injury. It was reported that EVs from human induced pluripotent stem cell [iPSCs] derived MSCs reduced the levels of the hepatic injury markers aspartate aminotransferase [AST] and alanine aminotransferase [ALT], multiple pro-inflammatory proteins including tumor necrosis factor [TNF]-alpha, interleukin [IL]-6, high mobility group box 1 [HMGB1] as well as the apoptotic markers caspase-3 and bax [51]. Moreover, EVs increased the expression levels of oxidative markers, such as glutathione [GSH], glutathione peroxidase [GSH-Px], and superoxide dismutase [SOD] [51]. In a toxicity-induced [n-acetyl-p aminophenol or hydrogen peroxide] *in vitro* injury model, EVs from hepatic stellate cells [HSC] LX-2 and HST-T6 also improved the viability, and inhibited the apoptosis, and reduced cleaved caspase-3 expression as well as the leakage of lactate dehydrogenase [LDH], ALT, and AST from hepatocytes in a dose-dependent manner [52].

Efficacy of EVs from human foetal tissue-derived MSCs and umbilical cord [UC]-MSCs in treating hepatic fibrosis has also been reported earlier [53, 54]. However, the role of MSC- derived EVs in regulating molecular pathways involved in hepatic fibrosis remains to be explored. Transdifferentiation or activation of quiescent hepatic stellate cells [qHSCs] into proliferative and fibrogenic myofibroblast like activated HSCs [aHSCs] has been identified as the key cause of liver fibrosis [55, 56]. EVs from MSCs are rich in paracrine factors and their role in immunomodulation has been widely acknowledged [57]. EVs from MSCs have been shown to possess the potential to regulate the activation of HSCs and to reduce hepatic fibrosis [53, 54]. It has been hypothesized that the presence of antifibrogenic miRNAs [miR-15a, miR-15b, miR-16, miR-29a, miR-195] in the EVs might have the potential to regulate the expression of genes involved in the HSCs activation such as collagen 1 [Colla1], actin alpha 2 [Acta2], transforming growth factor beta receptor 1 [Tgfb1], and tissue inhibitors of metalloproteinases [Timp1] [56, 58, 59]. Furthermore, expression of the targeted miRNAs in the EVs from MSCs could be induced and used as a potential therapeutic modality.

On the basis of all the research outcomes, it can be postulated that by inhibiting activation of hepatic stellate

cells, EVs could show its hepatoprotective effect. Meanwhile, by minimizing oxidative stress, modulating immune response and increasing hepatocyte proliferation EVs could contribute to liver regeneration.

### 2.3. Neuronal Regeneration

Acute ischemic stroke and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease are among the leading causes of death and disability in the world. The potential of cell-based regenerative therapy has been being studied in the last two decades. In recent years, the neuroregenerative potential of secretomes and EVs from different stem and progenitors has been explored [60-62].

Lower concentration of EVs [from murine adipose-derived [AD]-MSC have been found to protect human neuroblastoma SH-SY5Y cells and primary murine hippocampal neurons from chemically induced oxidative stress [61]. Similarly, increased remyelination and activated nestin-positive oligodendroglial precursors in the cerebral slice from C57BL/6 mice pups if exposed to a lower concentration of AD-MSC-EVs [61]. The authors concluded that AD-MSC-EVs could be beneficial in preventing neuro-inflammation and treating neurodegenerative disorders [61]. EVs from dental pulp stem cells [SHED] cultured on the laminin-coated three-dimensional alginate micro-carriers have been found to prevent apoptosis in dopaminergic neurons in the presence of the toxic dopamine derivative 6-hydroxy-dopamine [6-OHDA] [62].

In another study, an increased expression of miR-133b in the ipsilateral hemisphere of rats subjected to middle cerebral artery occlusion [MCAo] was seen when treated with MSCs. In the presence of ipsilateral ischemic tissue extracts from rats subjected to MCAo also induced the expression of miR-133b in MSCs and their exosomes *in vitro*. Furthermore, treatment with exosome-enriched fractions yielded from the MSCs induced expression of miR-133b in primary cultured neurons and astrocytes. The knock-down of miR-133b in MSCs confirmed that the increased miR-133b level in astrocytes is attributed to their transfer from MSCs. Exosome-enriched fractions from MSCs exposed to post-MCAo brain extracts also significantly increased the neurite branch number and total neurite length. This study revealed that MSCs communicate with brain parenchymal cells via exosomes and may regulate neurite outgrowth by transfer of miR-133b to neural cells [63]. Knock-in and knock-down of miR-133b further confirmed that neurological recovery following a stroke in rats was mediated by the transfer of miR-133b containing exosomes from MSCs [64].

Spinal cord injury [SCI] is a condition causing morbidity due to irreversible motor dysfunction and death as well. Several studies have reported the potential of EVs derived from different sources in improving motor function following SCI. Rong et al. [2019] reported the potential of neural stem cells derived EVs in reducing the extent of SCI and improving functional recovery of motor neurons in rats. This was further linked to the induced expression of autophagy proteins namely LC3B and beclin-1; boosted autophagosome formation; and reduced expression of apoptotic regulator Bax, apoptosis effector cleaved caspase-3, and pro-inflammatory paracrine factors TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 [65]. BMSC-EVs have also been reported to improve motor function in the rat by reducing

neuronal death and inducing neuronal survival and regeneration. Furthermore, BMSC-EVs improved blood spinal cord barrier integrity by downregulating NF- $\kappa$ B signaling in pericytes [66]. In another *in vivo* study, UCMSC-EVs were found to improve SCI by reducing pro-inflammatory cytokines expression and scar formation [67]. Notably, in a recent study, motor function improvement in SCI rats was linked to the presence of miRNA-29b in the exosomes [68]. Improved hind-limb movements and bladder control in chronic SCI dogs following multiple intravenous injections of BMMSC secretome further denote the neuro-regenerative potential of EVs [69].

Crossing the blood-brain barrier of therapeutic agents is the major challenge in treating neurodegenerative diseases. In this context, it has been demonstrated that EVs have the potential to cross the blood-brain barrier [70]. All these studies encourage further development of engineered exosomes as drug delivery vesicles or tracking tools for treating or monitoring neurological diseases.

## 2.4. Wound Healing

The prevalence of acute and chronic wounds is continuously increasing and being a major cause of morbidity. Acute wounds are caused by thermal injury, trauma, excision of extensive skin cancer, deep fungal and bacterial infections, vasculitis, scleroderma, pemphigus, toxic epidermal necrolysis, etc. Chronic wounds include diabetic ulcers, bedsores, arterial [ischemic] ulcers, and venous ulcers. These diseases increase the health care cost and thus the economic burden. Importantly, conventional treatments for these conditions are often ineffective [71]. Several *in vitro* and *in vivo* studies using EVs from different sources have been conducted to explore their potential in treating acute and chronic wounds.

New blood vessel formation is essential in wound healing. Crosstalk between endothelial cells, paracrine factors, and extracellular matrix [ECM] proteins play a vital role in the process of regeneration. Interestingly, dose-dependent uptake of BM-MSC-EVs by endothelial cells and an increased tube formation was reported *in vitro*. This was linked to activation of important wound healing signaling pathways [Akt, extracellular signal-regulated kinase [ERK], and signal transducer and activator of transcription [STAT3]] and induced expression of a number of paracrine factors [hepatocyte growth factor [HGF], insulin-like growth factor [IGF1], nerve growth factor [NGF], and SDF1] [72]. The presence of mRNA in the EPC-EVs has also been reported to induce angiogenesis in endothelial cells by regulating the phosphatidylinositol 3-kinase [PI3K]/Akt signaling pathway [73]. Among the EPCs subpopulations, EPCs with low activity of aldehyde dehydrogenase [Alde-Low EPCs] have been found to be biologically more active in repairing ischemic tissues [74]. Later it was reported that Alde-Low EPCs express a higher amount of hypoxia inhibition factor [HIF]-2 and its target gene CXCR4 [75]. Improved ability to repair an ischemic skin flap along with the significantly higher expression of CXCR4 and its ligand SDF1 in Alde-High EPCs in the presence of EVs from Alde-Low EPCs subpopulation was also reported [75]. These studies signify the role of EVs from Alde-Low EPCs in wound healing by regulating the cellular migration and blood vessel formation.

Preventing myofibroblast formation and accumulation is another major challenge in scar-free wound healing. EVs from umbilical cord-derived MSCs enriched in specific miRNAs such as miR-21, miR-23a, miR-125b, and miR-145 have been reported to play key roles in suppressing myofibroblast formation by inhibiting the transforming growth factor-beta 2 [TGF- $\beta$ 2]/SMAD2 pathway [76]. Significantly accelerated re-epithelialization, and increased expression of CK19, proliferating cell nuclear antigen [PCNA], collagen I [compared to collagen III] in deep second-degree burned rat skin treated with hUC-MSC-EVs was also shown. Induced wound re-epithelialization and reduced heat stress-induced apoptosis of cells in the hUC-MSC-EVs treated rat were further connected to the activation of Wnt/catenin and Akt signaling pathways respectively [77]. Another study was conducted to reveal the potential of MSC-EVs in controlling overcrowding and dysplasia following stem cells expansion after a regenerative response. The researchers have reported that hUC-MSC-EVs contain 14-3-3 $\zeta$ . Under high cell density conditions, 14-3-3 $\zeta$  regulates the binding of YAP and phosphorylated large tumor suppressor kinase [LATS] by forming a complex to promote the phosphorylation of yes-associated protein [YAP] followed by inhibition of Wnt4/catenin signaling cutaneous regeneration [78]. Outcomes of these studies indicate that hUC-MSC-EVs not only induce wound healing by activating Wnt/catenin signaling but also regulate YAP to control cutaneous regeneration.

## 2.5. Bone Regeneration

The incidence of bone disorders has been increasing worldwide underlining the necessity of regenerative therapies for bone tissue. Like in other tissues, cell fate and bone regeneration are also influenced by EVs secreted by cells residing within the injured tissue. Lineage-specific expression of miRNA in ESCs in the presence of preosteoblast-EVs has been reported earlier [79]. Studies have also shown that EVs collected from both BM-MSCs regular culture medium and osteogenic differentiation medium have the potential to induce osteogenic differentiation of naive BM-MSCs [80, 81]. However, the lineage-specific differentiation of naive BM-MSCs *in vitro* and *in vivo* was higher in the presence of EVs isolated from osteogenic differentiation media [81]. EVs collected from both cell culture conditions have revealed a binding capacity to matrix proteins such as type I collagen and fibronectin [81]. These results suggest the potential future use of EVs to pretreat autologous cell populations prior to transplantation to improve the bone regenerative outcomes [81].

In another study, fracture healing in CD9 $^{-/-}$  mice was accelerated by BM-MSCs derived EVs, despite having the lower levels of the bone repair-related cytokines such as monocyte chemoattractant protein-1 [MCP-1], MCP-3, and SDF1 compared to the levels in BM-MSCs condition medium and EVs free condition medium [82]. This result highlights the role of miRNAs in accelerating the bone repairing capability of EVs.

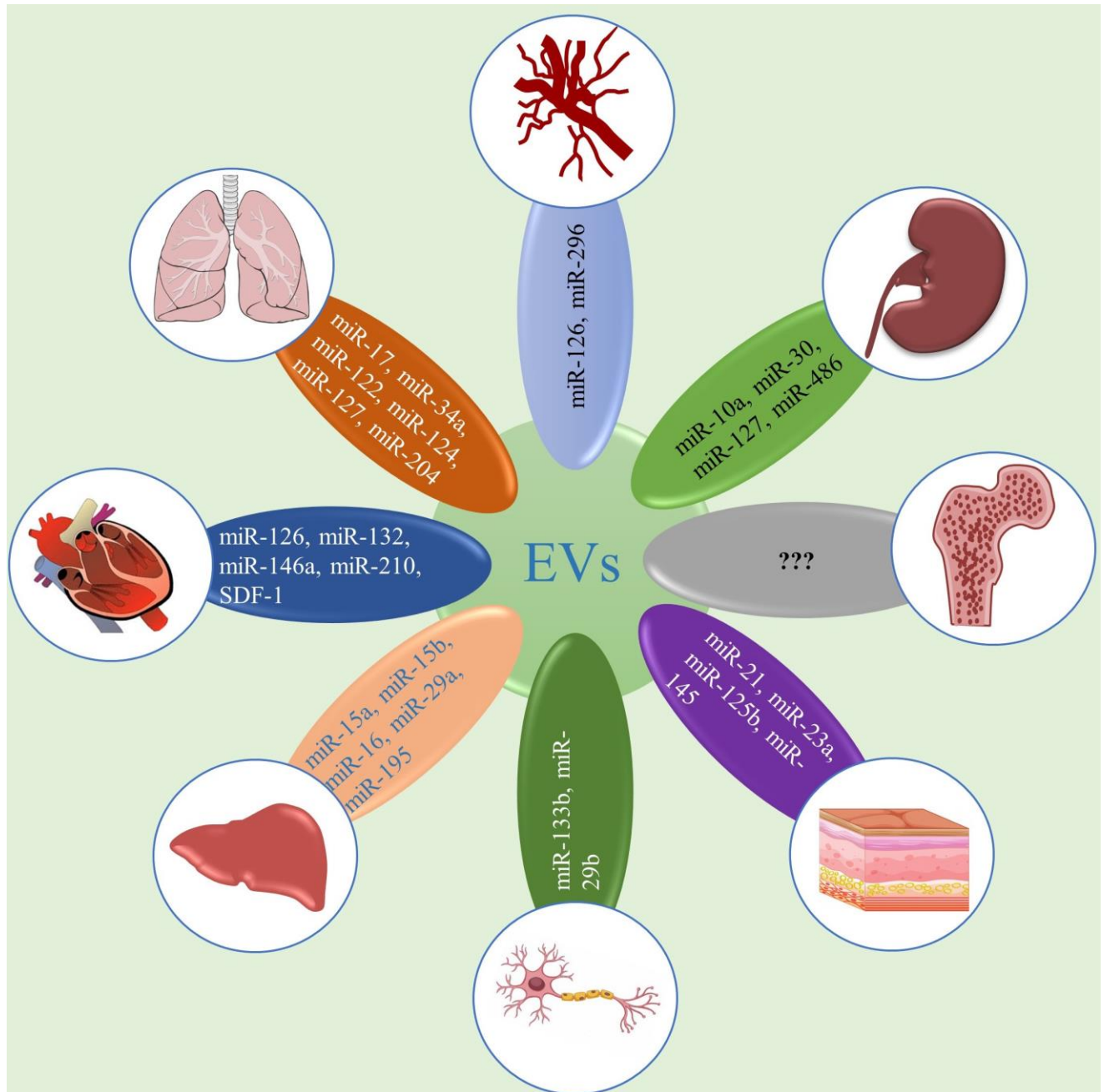
## 2.6. Graft versus Host Disease

Several studies reported MSCs as being immune evasive rather than immune privileged [83, 84]. Although there are controversies regarding the immunomodulatory properties of MSCs, several studies have acknowledged their potential in preventing life-threatening graft versus



host disease [GVHD] [85-87]. Similar to the regenerative and immunomodulatory potentials of MSCs themselves, immunomodulatory properties of MSC-derived EVs have also received remarkable attention [88-90].

IFN- $\gamma$  from patient-derived natural killer [NK] cells in the presence of MSC-EVs have also been reported in a clinical study [93]. Notably, the immunomodulatory properties of EVs are linked to the internalization of the EVs by the



**Figure 2** Components of extracellular vesicles for tissue or organ-specific regenerative therapy.

In an *in vivo* study, the infusion of hUC-MSC-EVs into mice following allogeneic hematopoietic stem cell therapy [HSCT] showed a prophylactic effect and significantly reduced the mortality of the recipient mice. These EVs significantly reduced the numbers of cytotoxic/suppressor T cells and the expression of paracrine factors [IL-2, TNF- $\alpha$ , and interferon [IFN]- $\gamma$ ] in the serum. Simultaneously, it increased the ratio between CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup> T cells and elevated the expression levels of IL-10. [91]. In another study, BM-MSC-EVs have been shown to induce apoptosis of CD3<sup>+</sup> cells including the CD4<sup>+</sup> subpopulation. Furthermore, it increased proliferation and apoptosis of Tregs, the Treg/Teff ratio, and the levels of the immunosuppressive cytokine IL-10 in cell culture supernatants [92]. Reduced expression of IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$  from the patient-derived PBMC or TNF- $\alpha$  and

cells [94].

There are controversies on the reduction of GVHD by MSC-EVs [95]. However, the positive outcomes of the most of the studies necessitate to study the compositions [proteins, mRNA and miRNA] and immunomodulatory properties of EVs to find out the key components involved in the innate and adaptive immune regulation and to translate their uses in preventing GVHD [87, 96].

## 2.7. Kidney Regeneration

The role of MSCs in preventing acute kidney injury by ameliorating renal dysfunction and repairing tubular damage has been acknowledged in several studies [97, 98]. However, the regenerative outcomes were facilitated by the paracrine effects of MSCs rather than their homing and



differentiation to target cells [99]. In 2013, Tomasoni et al. reported that BM-MSC-EVs horizontally transfer IGF-1R mRNA to the tubular cells which enhanced tubular cell sensitivity to locally produced IGF-1 and prevent cisplatin-induced damage of proximal tubular cells *in vitro* [100]. The role of hWJ-MSC-EVs in ameliorating ischemia/reperfusion-induced acute renal injury by inhibiting mitochondrial fission through delivering and restoring miR-30 expression in renal tissue has also been reported [101]. In another study, MSCs engineered with miRNAs [miR-10a, miR-127, and miR-296] maintained their pro-regenerative effects macrophage-mediated inflammation following acute ischemia/reperfusion-induced renal injury is one of the major cause of renal damage [102, 103]. Recently, CCR2 positive MSC-EVs prevented ischemia/reperfusion-induced renal injury by suppressing the functions of C-C motif chemokine ligand [CCL2] that usually recruit or activate macrophage [104]. Thus, EVs from different cell sources could be developed as a therapeutic tool to treat kidney injuries.

## 2.8. Human Islet Vascularization

Pancreatic islet transplantation is considered as one of the treatment modalities in type 1 diabetes patients whose blood glucose level is difficult to control [105]. However, poor graft vascularization limits the functional benefits of islet transplantation. Cantaluppi et al. [2012] have shown enhanced human islet vascularization in response to EPCs-EVs *in vitro* and *in vivo*. The authors have shown that following internalization of EPC-EVs by islet endothelium and beta-cells, insulin secretion, survival, and revascularization of transplanted islets in SCID mice was induced. EPC-EVs induced *in vitro* proliferation, migration, resistance to apoptosis, and neovascularization of islet endothelial cells. The authors have further linked the presence of miR-126 and miR-296 in EPC-EVs to an induced revascularization and insulin secretion potential of EPC-EVs in islet endothelial cells. Microarray analysis of islet endothelium further confirmed EPC-EVs mediated upregulation of mRNAs that are involved in endothelial proliferation, differentiation, and angiogenesis. Activation of PI3K-Akt and eNOS signaling pathways which are vital for vascularization was also reported in EPC-EVs treated islet endothelium [106].

## 2.9. Lung Repair and Regeneration

Studies also reported the influence of lung derived EVs on the modification of fate and function of marrow cells [107, 108]. These results suggest the use of lung cell-derived EVs for priming the stem or progenitor cells prior to transplantation within a regenerative therapy. Furthermore, MSC-EV containing miR-34a, miR-122, miR-124, and miR-127 reported to improve the pulmonary hypertension in mice [109]. The potential of human MSC-EVs in protecting the lung from *E. coli* endotoxin-induced acute lung injury in mice by expressing KGF mRNA in the injured alveolus has also been reported earlier [110].

## 3. LIMITATIONS OF USING EVS IN REGENERATIVE THERAPY

Within regenerative medicine, stem cells are being considered as a promising tool to treat incurable and difficult to treat diseases and conditions. However, in recent years, EVs have received substantial attention as potential cell-free surrogates to stem cell transplantation. EVs contain mainly miRNAs, mRNAs, and proteins that regulate bi-

directional cell-cell communication. EVs can be used directly to treat any diseases or to precondition stem cells prior to transplantation to improve their regenerative or lineage-specific differentiation potential. Despite having regenerative potential, the composition of EVs varies vastly which limits its successful and replicable therapeutic use. Moreover, bio-distribution of endo- and exo-genously administered EVs have not been studied extensively [111]. Another concern is related to the use of xenogeneic serum and feeder cells in the production of EVs. It has been reported that hESCs maintained with xenogeneic feeder layer cells secrete a higher amount of particular proteins [annexin II, beta-actin, guanine nucleotide-binding protein, enolase 1, and cofilin] secrete EVs with a cargo involved in cellular metastasis and immortality which is usually present in invasive cancers. and [112]. Furthermore, like stem cells, xenogeneic serum, and feeder cells could contaminate the EVs with xeno-antigens that could affect their regenerative potential [112]. Finally, the safety of EVs, delivery mechanisms, potency of the EVs, and regulatory issues [manufacturing, standardizing, and up-scaling] need to be extensively studied for their successful use in regenerative medicine.

## CONCLUSION

The use of EVs as cell-free therapeutics will help to minimize a lot of limitations related to the cell-based therapy. By analyzing the results from *in vitro* and *in vivo* studies on EVs, we attempted to categorize them on the basis of their contents and/or sources while considering their targeted regenerative potential. This categorization and enriching it with updated research outcomes will be very effective in selecting EVs to treat a particular tissue, organ, or ailment. Moreover, it will help the researcher to make decision in selecting individual or group proteins or genetic materials whose expression needed to be enhanced in EVs for targeted therapy. EVs are very small in nature that have the potential to cross the blood-brain barrier. Hence, beside carrying genetic materials or proteins they could be used as a carrier of bioactive drugs or components in near future. However, despite the high therapeutic potential, any future EV-based therapy must adhere to strict standards with clear safety and risk profile, and a defined mode of action. This could not only lead to a development of affordable cell-free therapeutics but also prevent unproven commercial direct-to-patient exploitation of EV-based therapies that could harm the patients and the credibility of the field.

## LIST OF ABBREVIATIONS

Acta2	= actin alpha 2
AD	= adipose derived
aHSCs	= activated HSCs
Akt	= protein kinase B
ALT	= alanine aminotransferase
AST	= aspartate aminotransferase
ATP	= adenosine triphosphate
BM	= bone marrow
BMSC-EVs	= BM stem cells derived EVs
CCL	= C-C motif chemokine ligand
CD	= cluster of differentiation
CK19	= cytokeratin 19
Colla1	= collagen I
CPCs	= cardiac progenitor cells
CXCR	= C-X-C motif chemokine receptor

ECM	= extracellular matrix
EPCs	= Endothelial progenitor cells
ERK	= extracellular signal-regulated kinase
EVs	= extracellular vesicles
GSH	= glutathione
GSK-3	= Glycogen synthase kinase-3
GSK-3	= Glycogen synthase kinase-3
GVHD	= graft versus host disease
H <sub>2</sub> O <sub>2</sub>	= hydrogen peroxide
HGF	= hepatocyte growth factor
HIF	= hypoxia inhibition factor
HLSC	= human adult liver stem cells
HMGB1	= high mobility group box 1
HSC	= hepatic stellate cells
HSCs	= hematopoietic stem cells
HSCT	= hematopoietic stem cell therapy
HUVEC	= human umbilical vein endothelial cells
hWJ	= human Wharton jelly
IGF-1	= insulin like growth factor
IL	= interleukin
iPSCs	= induced pluripotent stem cells
KGF	= keratinocyte growth factor
LATS	= large tumor suppressor kinase
LDH	= lactate dehydrogenase
MCAo	= middle cerebral artery occlusion
MCP-1	= monocyte chemotactic protein-1
miRNA	= microRNA
MSC-EVs	= MSC derived EVs
MSCs	= mesenchymal stem cells
NADH	= nicotinamide adenine dinucleotide
hydrogen	
NF- $\kappa$ B	= nuclear factor kappa beta
NGF	= nerve growth factor
NK	= natural killer
p-C-JNK	= C-Jun N-terminal kinase
PCNA	= proliferating cell nuclear antigen
PI3K	= phosphatidylinositol 3-kinase
Px	= peroxidase
qHSCs]	= quiescent hepatic stellate cells
SCI	= spinal cord injury
SCID	= severe combined immunodeficiency
SDF-1	= stromal cell derived factor 1
SHED	= stem cells from human
extracted/exfoliated mesenchymal stem cells	
SOD	= superoxide dismutase
STAT	= signal transducer and activator of
transcription	
Tgfr	= TGF- $\beta$ receptor
TGF- $\beta$	= transforming growth factor-beta
Timp	= tissue inhibitors of metalloproteinases
TNF	= tumor necrosis factor
UC	= umbilical cord
UCMSC-EVs	= UC MSCs derived EVs
VEGF	= vascular endothelial growth factor
YAP	= yes-associated protein

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## Conflict of Interest

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## CREDIT AUTHOR CONTRIBUTION STATEMENT

N.H. contributed to the conceptualization, visualization, and writing [original draft, review, and editing] of the manuscript. D.W. and V.G. contributed to the conceptualization, and writing [review, and editing] of the manuscript. N.H.A.K. contributed to the conceptualization and writing [original draft, review, and editing] of the manuscript. L.M.N. contributed to the writing [review, and editing] of the manuscript. All authors approved the final version of the article to be submitted and all authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## OCRID

Nazmul Haque: <https://orcid.org/0000-0002-8191-8993>

Darius Widera: <https://orcid.org/0000-0003-1686-130X>

Vijayendran Govindasamy: <https://orcid.org/0000-0002-5955-3553>

Pratiwi Soesilawati: <https://orcid.org/0000-0002-1141-5645>.

Noor Hayaty Abu Kasim: <https://orcid.org/0000-0002-8889-842X>

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