



Review

# Therapeutic Potential of Extracellular Vesicles in Aging and Age-Related Diseases

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**Abstract:** Aging is associated with an alteration of intercellular communication. These changes in the extracellular environment contribute to the aging phenotype and have been linked to different aging-related diseases. Extracellular vesicles (EVs) are factors that mediate the transmission of signaling molecules between cells. In the aging field, these EVs have been shown to regulate important aging processes, such as oxidative stress or senescence, both in vivo and in vitro. EVs from healthy cells, particularly those coming from stem cells (SCs), have been described as potential effectors of the regenerative potential of SCs. Many studies with different animal models have shown promising results in the field of regenerative medicine. EVs are now viewed as a potential cell-free therapy for tissue damage and several diseases. Here we propose EVs as regulators of the aging process, with an important role in tissue regeneration and a raising therapy for age-related diseases.

**Keywords:** intercellular communication; extracellular vesicles; stem cells; age-related diseases



**Citation:** Sanz-Ros, J.; Mas-Bargues, C.; Romero-García, N.; Huete-Acevedo, J.; Dromant, M.; Borrás, C. Therapeutic Potential of Extracellular Vesicles in Aging and Age-Related Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 14632. <https://doi.org/10.3390/ijms232314632>

Academic Editor: Denis N. Silachev

Received: 29 October 2022

Accepted: 22 November 2022

Published: 23 November 2022

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## 1. Introduction

Stem cell therapy has been the leading framework in regenerative medicine. Owing to their self-renewal capacity, exogenous stem cells should be able to regenerate different tissues after damage. Mesenchymal stem cells (MSCs), due to their easy isolation, culture, and low immunogenicity, have been the most studied type of cells for their application in a clinical setting [1]. Historically, the main hypothesis was that stem cells should have the ability to integrate into damaged tissue and repopulate it through asymmetric division. However, more recent studies have shown that allogeneic MSCs have a limited potential for integration into a tissue, as most commonly, less than 1% of the transferred cells survive more than a week in the host organism [2–5]. Despite these facts, MSCs therapies have been demonstrated to be beneficial for many conditions and improve the regeneration of several tissues, suggesting that the most significant effects of these cells are mediated by paracrine mechanisms [6,7]. Thus, the secretome of MSCs has been positioned as a potential therapy for tissue repair and regeneration.

Among all the factors that stem cells release to the extracellular environment, both in vivo and in vitro, extracellular vesicles (EVs) have emerged as a very interesting choice. These lipid bilayer vesicles are released by virtually every cell type and contain a myriad of molecules that are thought to be responsible for their role in the communication between cells, through the exchange of proteins, nucleic acids, or lipids [8,9]. There are many types of EVs, such as exosomes, microvesicles, ectosomes, oncosomes, or apoptotic bodies, these are classified attending to their biological origin and role [8]. However, this classification is not the most convenient due to the lack of methods that can separate specifically the different subtypes of EVs. The most used methods for isolating and separating EVs rely on their size (i.e., differential ultracentrifugation, filtration, size exclusion chromatography, or

high molecular weight filters). Other methods are based on the differences in EVs density (density gradient) or specific surface molecules (immunoaffinity isolation techniques). The International Society for Extracellular Vesicles proposes a classification based on the size of the vesicles, distinguishing small extracellular vesicles (<200 nm in diameter) from other medium and larger vesicles [9]. Although small extracellular vesicles are probably the most studied, here we will describe findings related to all types of EVs, regardless of the nomenclature used by the authors of each study.

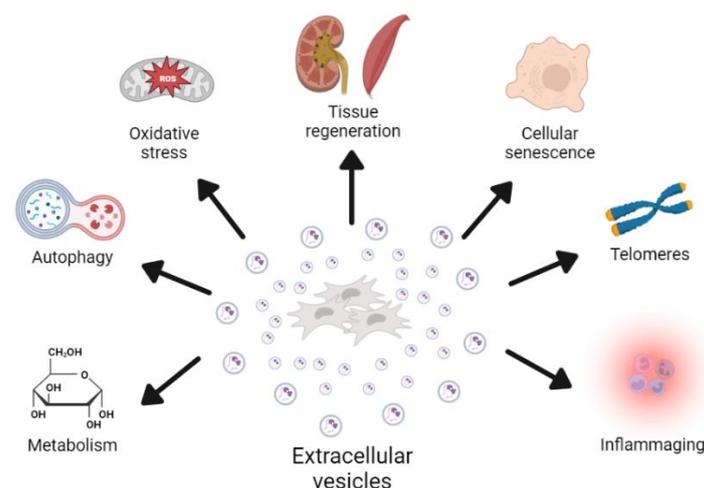
The use of EVs adds several advantages over the use of stem cells to improve tissue regeneration. EVs are more stable, their dosage is easier, they do not have a risk of aneuploidy, and they have a lower incidence of immune rejection [7]. EVs from several types of stem cells have demonstrated their potential to improve regeneration in many different tissues after induced damage, such as the kidney, liver, heart, or brain [10].

Aging is characterized by a loss of tissue regenerative capacity. All the stem cells' niches that are present in an adult organism lose the ability to regenerate properly, both in basal conditions and after damage, with a lower number of potential divisions over a longer period [11]. As aging progresses, progenitor cells responsible for damaged tissue regeneration suffer molecular traits that affect their ability to replicate, such as DNA damage accumulation, telomere attrition, or senescence [12]. EVs from stem cells and other cell types are now being studied as potential regulators of many processes associated with aging, such as cellular senescence, oxidative stress, telomere dysfunction, autophagy inefficiency, inflammation, and metabolic dysregulation.

In this review, we aim to point out the idea that EVs from different sources such as stem cells could be beneficial for the aging-associated decline in tissue function and age-related diseases, aiding tissue regeneration through different pathways.

## 2. Role of EVs in the Aging Process

Aging is characterized by molecular and cellular traits that are thought to be the drivers of this process, which ultimately lead to tissue dysfunction and an increased risk of death [12,13]. EVs from different cells have been described as factors that can modify these aging-associated processes (Figure 1). Here, we will describe some of the last findings regarding the role of EVs in aging.



**Figure 1.** Graphical explanation of the role of EVs in the modulation of multiple processes associated with aging. The concrete effects of these processes are described in the manuscript, and EVs have been shown to participate in both directions. Depending on cell source and condition, EVs have beneficial or deleterious effects on age-related processes.

### 2.1. Cellular Senescence

Cellular senescence is a physiological process that some cells suffer when exposed to different types of damage, such as DNA damage, oncogene activation, telomere attrition,

or radiation [14]. These cells enter a cell cycle arrest that prevents their proliferation as a tumor suppressor mechanism. However, the accumulation of these cells during aging, due to increased production and a decreased clearance by the immune system, has been linked to several aging processes and pathologies [15]. An important contributor to the deleterious effect of senescent cells is the accumulation of different factors in the extracellular milieu, the senescence-associated secretory phenotype (SASP) [16]. These factors have been described as drivers of paracrine senescence, cancer growth, and inflammation, among others [17]. As players in intercellular communication, EVs are now regarded as important factors of the SASP [18], with an important role in the deleterious effects of senescence, referred to as evSASP [19,20]. The release of EVs is typically increased in senescent cells, and its content changes when cells become senescent, suggesting an important mechanism to target in cellular senescence [20,21].

Some studies have shown a promising therapeutic effect of EVs from healthy, non-senescent cells, such as stem cells, in the modulation of senescence. EVs from healthy cells can decrease senescence both *in vivo* and *in vitro* [22–24] through several proposed mechanisms, such as the transmission of miRNAs [22,24], antioxidant enzymes [23], or metabolic changes [25]. Due to their immunomodulatory roles, EVs can act through the modulation of the SASP, decreasing the pro-inflammatory changes in the microenvironment induced by the accumulation of senescent cells. One of the proposed mechanisms that might explain the effects of EVs on senescence is viewing EVs from healthy cells as senomorphics, factors that can decrease the burden of senescent cells, not by directly inducing their selective apoptosis as senolytics but by modifying the extracellular environment and inhibiting the SASP [24,26].

## 2.2. Oxidative Stress

Oxidative stress, and more concretely, the fine control of the redox status of a cell or a tissue, is thought to contribute to the aging process and age-related morbidities [27,28]. The balance between oxidative and reductive molecules is crucial to the proper functionality of the cells. During aging, dysregulation of these control mechanisms occurs, with some tissues tending to accumulate oxidative damage in several macromolecules, mainly lipids, proteins, or DNA. This damage contributes to the development of age-related pathologies, such as Alzheimer's [29] or frailty [30,31]. EVs are now viewed as important factors that regulate the redox status through the transfer of molecules with antioxidant properties between cells, protecting against oxidative damage in several models of acute damage and during aging [23,24,32]. The different conditions associated with the culture of cells can modify EVs release, and the content of the oxygen tension in the culture is of the utmost importance [33,34]. EVs from cells cultured under a physiological oxygen tension show increased therapeutic effects in some models of tissue damage, with changes in their content and release, suggesting that oxygen availability is an important mechanism that modulates EV biogenesis and content [35,36].

## 2.3. Telomere Dysfunction

Telomere shortening and damage, as well as dysregulation of the machinery that controls the structure of telomeres, are important players in the aging process and are tightly related to other processes of aging, such as senescence or genomic instability [37,38]. In these terms, EVs have been shown to contain short non-coding telomere RNA transcripts that can induce the release of inflammatory cytokines in recipient cells, linking telomere dysfunction and inflammation through EV release [39]. EVs from stem cells have been shown to increase telomerase activity in a model of osteoporosis in mice. Moreover, EVs have been described as factors that regulate telomere-related bystander effects in irradiated breast cancer cells. EVs from irradiated cells decreased telomerase activity in recipient cells, with shorter telomeres over time in these cells [40]. RNase treatment suppressed these effects, suggesting the role of RNAs contained in EVs in the regulation of telomere function.

In a more recent study, telomere vesicle transfer from antigen-presenting cells elongated telomeres in recipient T cells independently of telomerase activity [41].

#### 2.4. Autophagy

The correct regulation of autophagy is key to maintaining proteostasis during aging, and a loss of this regulatory mechanism has been linked to aging and age-related diseases [42,43]. During aging, there is an accumulation of damaged components of the proteome, both intracellular and extracellular, such as misfolded or aggregated proteins [12]. Trying to improve autophagy signaling is an interesting approach to treating age-related diseases, such as Parkinson's or Alzheimer's [44]. Autophagy is closely related to the biogenesis of extracellular vesicles, as exosomes are included in multivesicular bodies before their release [8], and some autophagy-related proteins, such as ATG5, have been shown to regulate exosome biogenesis [45]. EVs from different types of MSCs have been demonstrated to promote the autophagic flux in several disorders associated with aging. In a model of diabetic nephropathy, EVs from ADSCs inhibited excessive mTOR activation, leading to increased autophagy [46,47]. A similar effect of improved outcomes due to autophagy activation has been observed in liver fibrosis [48] and spinal cord injury [49]. Therefore, the modulation of autophagy by EVs, and vice versa, may serve as a therapeutic tool in age-related conditions and the maintenance of proteostasis.

#### 2.5. Inflammation

One of the most studied effects of EVs is their potential role in regulating the immune system, as EVs from damaged cells usually induce an activation of the immune system, leading to the release of pro-inflammatory factors [50]. On the other hand, EVs from MSCs are now viewed as significant anti-inflammatory and immunomodulatory factors [51,52]. MSCs themselves have been studied for a long time as promising therapeutics for inflammatory diseases, as they have been shown to decrease the pro-inflammatory activity of several cell types, such as neural, vascular, or osteochondral [53]. During aging, an imbalance between pro and anti-inflammatory factors occurs, leading to an accumulation of pro-inflammatory factors in the extracellular environment that accompanies normal aging and that has been linked to several age-related diseases [54]. This chronic inflammation has been termed "inflammaging", and the interventions targeting this process have beneficial effects for age-related conditions [55,56]. As stated before, the beneficial effect of EVs observed in many models of disease, such as myocardial infarction, stroke, chronic kidney disease (CKD), or liver injury, is typically accompanied by a suppression of pro-inflammatory cytokines, with an increase in anti-inflammatory molecules. In physiological aging, the treatment with EVs has been shown to decrease inflammatory cytokines that are associated with aging, such as IL-6, IL-1 $\beta$ , or TNF- $\alpha$  [24,26,57,58]. As we have described, EVs are important factors of the SASP that lead to paracrine senescence and contribute to the chronic inflammation observed during aging [20]. One of the proposed mechanisms through which EVs from healthy cells could decrease inflammation and senescence is the inhibition of the SASP, acting as "senomorphics", linking their effect both in cellular senescence and inflammation [24,26].

#### 2.6. Metabolism

One of the most conserved mechanisms in evolution that controls aging and longevity is metabolism, mainly through several pathways related to nutrient sensing and growth, such as insulin/IGF-1, mTOR, AMPK, or sirtuins [59–62]. In this sense, manipulations that target these pathways have been demonstrated to have a wide effect on longevity and health span (i.e., caloric restriction or mTOR inhibitors) [63–65]. EVs have been shown to induce profound metabolic changes in recipient cells; for example, EVs from obese mice can increase insulin resistance and dyslipidemia in healthy mice through the transfer of miRNAs [66,67]. Moreover, miRNAs that suffer age-associated changes in plasmatic EVs are important regulators of these metabolic pathways [68]. As aging progresses, the content

of nicotinamide phosphoribosyltransferase (NAMPT) in circulating EVs decay, and the restoration of circulating levels of NAMPT have shown to improve health span and extend lifespan in mice, mainly through the increase in NAD<sup>+</sup> biosynthesis in old tissues [25]. In another study, authors observed that plasmatic EVs from young mice induce a decrease in mTOR and IGF1R in the liver and lungs of old mice [58]. All these data suggest that EVs play a role in metabolic regulation, with important effects on age-related pathways.

### 3. Pro-Regenerative Effects of EVs

Aging is characterized by decreased tissue repair and regeneration; thus, we propose that EVs may be useful for treating diseases associated with anomalous repair after damage. EVs from different sources have been applied in preclinical models of tissue damage. The most used source in these studies is MSCs, probably due to their simpler isolation and culture techniques. The site of EV injection varies between studies, with the majority injecting them intravenously or at the site of the damage whenever possible. Here we will briefly describe the effects on several damaged tissue models of various types of EVs. A summary of the pathways involved in the beneficial effects of EVs in tissue regeneration is shown in Table 1.

**Table 1.** Outline of the pathways involved in the pro-regenerative effects of EVs in different tissues. PI3K: phosphatidylinositol-3-kinase; AKT: protein kinase B; WNT: wntless-related integration site; JNK: c-Jun N-terminal kinase; ATM: ATM serine/threonine kinase; IGF: insulin-related growth factor; HGF: hepatic growth factor; FGF: fibroblast growth factor; mTOR: mammalian target of rapamycin; RPTOR: regulatory-associated protein of mTOR; TGF- $\beta$ : transforming growth factor  $\beta$ ; CCL2: chemokine (C-C motif) ligand 2.

| References | Tissue            | Effect  | Pathway Involved  |
|------------|-------------------|---|---|
| [69]       | Brain             | Neuroprotective effect in ischemia and traumatic brain injury | Activation of PI3K/AKT pathway and calcium oscillations         |
| [70]       | Brain             | Decrease in neuronal apoptosis and motor recovery             | Activation of Wnt/ $\beta$ -catenin signaling                   |
| [71]       | Heart             | Enhanced myocardial viability in ischemia/reperfusion         | Activation of PI3K/AKT pathway and reduced phosphorylated-c-JNK |
| [10,72,73] | Endothelial cells | Improved angiogenesis   | Activation of VEGF signaling and PI3K/AKT, repression of ATM    |
| [74]       | Muscle            | Prevention of muscle damage in hind limb ischemia             | Activation of Neuregulin-1                                      |
| [74]       | Muscle            | Improved myogenesis   | Increased IGFs, HGFs and FGFs                                   |
| [75]       | Muscle            | Improved muscle regeneration in aging                         | Activation of Klotho pathway                                    |
| [76]       | Bone              | Decrease in radiation-induced bone loss                       | Activation of Wnt/ $\beta$ -catenin signaling                   |
| [77]       | Bone              | Improved bone regeneration                                    | Activation of AKT/mTOR pathway                                  |
| [78]       | Lung              | Amelioration of acute lung injury                             | Induction of RPTOR/mTOR pathway                                 |
| [79]       | Lung              | Antifibrotic effect in a model of pulmonary fibrosis          | Inhibition of TGF- $\beta$ -Wnt crosstalk                       |
| [80]       | Kidney            | Decreased kidney injury in ischemia/reperfusion               | Inhibition of CCL2 pathway                                      |
| [81]       | Liver             | Amelioration of hepatic ischemia/reperfusion injury           | Inhibition of MEK/ERK pathway                                   |

### 3.1. Effects of EVs on the Nervous Tissue

Nervous tissue has a limited regenerative capacity upon several types of damage, both in peripheral nervous tissue and in the central nervous system [82]. Stem cell-derived EVs have been shown to improve regeneration of the nervous tissue after some types of damage in several animal models, such as peripheral nerve denervation [83,84], trauma [85], and stroke [86,87], with a neuroprotective effect on models of hypoxic-ischemic encephalopathy [69,88]. These EVs seem to exert this role through different processes and pathways associated with nerve regeneration. For example, in a recent study, Turovsky et al. demonstrated that EVs from MSCs exert a neuroprotective effect in a mouse model of ischemia through the modulation of phosphatidylinositol 3-Kinase/AKT signaling and calcium oscillations [69]. Moreover, EVs from different sources can induce changes in the glial cells, increasing the percentage of proliferating Schwann cells by 20% and increasing the myelinating potential of oligodendrocytes [89]. EVs also induce protective effects on the neurons themselves, improving neurogenesis and angiogenesis [87], doubling neurite length and increasing branch number by 75% [86], and reducing neuron apoptosis and neuroinflammation [70].

### 3.2. Effects of EVs on the Cardiovascular System

The models used to test the regenerative effects of EVs from different types of stem cells have been mainly models of myocardial infarction and ischemia-reperfusion injury. In these studies, EVs have been demonstrated to improve cardiac tissue repair, inhibiting the adverse remodeling that typically occurs after myocardial ischemia [71,90]. Cardiomyocytes are post-mitotic cells that do not regenerate after damage; the presence of resident cardiac stem cells in heart tissue is still debated [91]. EVs promote the regeneration of tissue through different pathways. It has been described that these EVs improve cardiomyocyte viability, reduce infarct size by 50% [71], decrease the apoptosis of these cells [92], and increase the capacity to revascularize the damaged tissue through improved angiogenesis [72]. EVs encapsulated in hydrogels have been demonstrated to decrease the excessive fibrosis and upregulation of pro-inflammatory factors, which are linked to a defective repair of cardiac tissue [93].

Endothelial cells are involved in the vascularization of every organ, and adequate angiogenesis is crucial to the regenerative process [94]. In this regard, EVs from stem cells and other cell types have been linked to improved angiogenesis in many tissue damage models, thus increasing the tissue regeneration potential of the tissue [73].

### 3.3. Effects of EVs on the Musculoskeletal System

EVs from several types of stem cells have been demonstrated to improve the regeneration of different tissues of the musculoskeletal system. EVs from muscle progenitor cells improve myogenesis both in vitro and in vivo [95]. In muscle damage models, such as ischemia [96], torn rotator cuffs [97], or muscle laceration [74], EVs can improve regeneration through increased angiogenesis, satellite cell activation [74], and decreased inflammation and fibrosis [95]. In the field of aging, EVs from the serum of young mice have shown an effect on improving muscle regenerative capacity in a model of cardiotoxin damage in old mice [75]. EVs from MSCs can improve and accelerate bone healing in models of fracture [98] or radiation-induced bone damage [76], inducing osteogenic cell proliferation and bone angiogenesis, leading to an increase in new bone area formation of 40% [77,99]. Similar effects have been observed in models of cartilage damage, where EVs from MSCs improve cartilage regeneration when injected intraarticularly [100].

### 3.4. Effects of EVs on Damaged Lungs

Some models of acute lung injury, such as hyperoxia [101], severe pneumonia [102], or endotoxin [103], have been developed to test the effect that EVs from stem cells have on lung tissue regeneration and repair. In these studies, EVs from different sources have been demonstrated to improve tissue function, reducing inflammation and edema [104], oxidative stress, senescence, and fibrosis [78,79]. More specifically, EVs attenuated pulmonary fibrosis by 33% in a model of bleomycin-induced fibrosis, ameliorating myofibroblast division and collagen accumulation and inducing structural changes in the alveolar structure [79].

### 3.5. Effects of EVs on Damaged Kidney

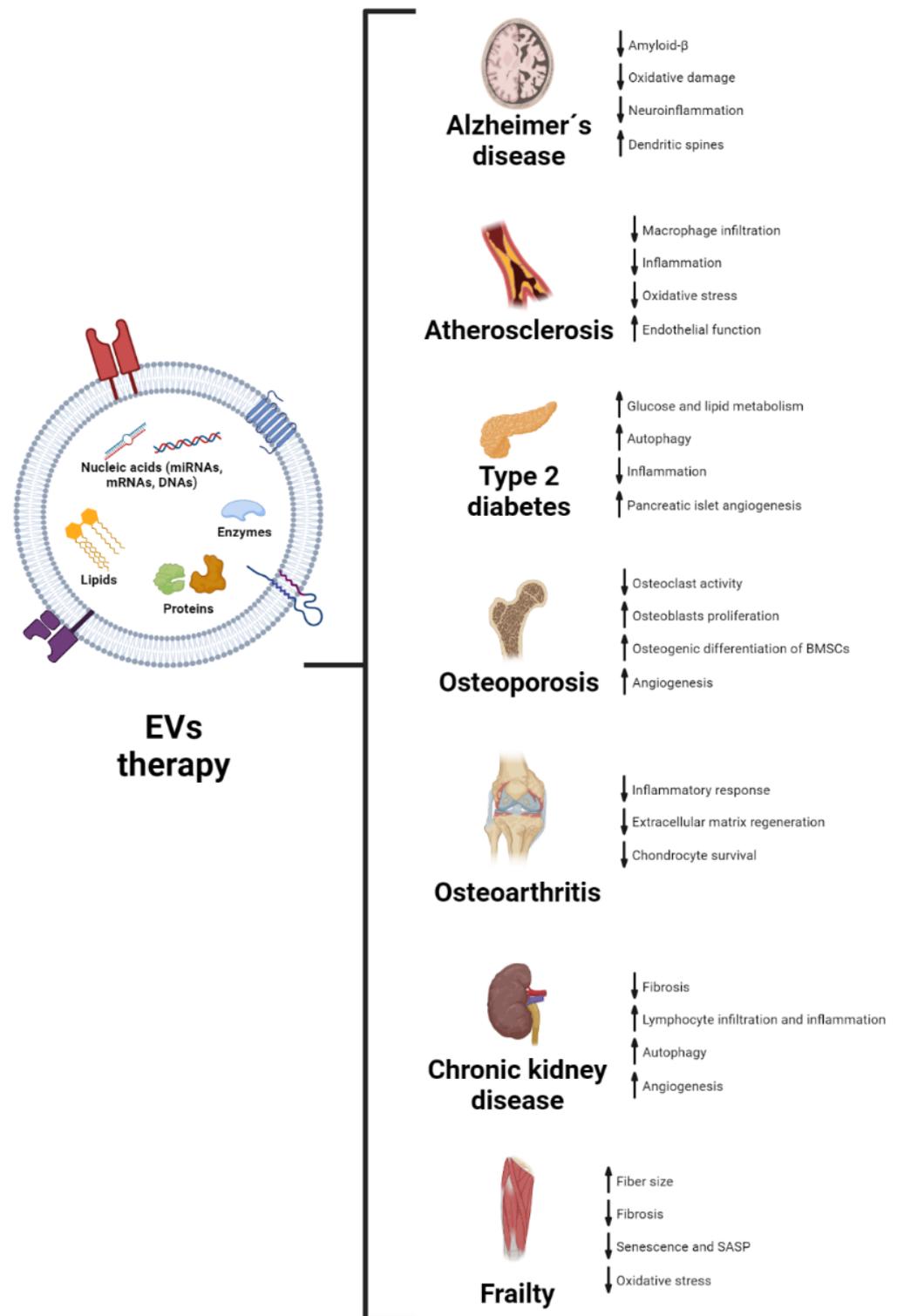
The functional unit of kidneys, the nephron, is not able to fully regenerate after damage in adult kidneys, as they cannot form new glomeruli [105]. However, tubular epithelial cells can repopulate the damaged tissue after damage [106]. EVs from stem cells have been shown to improve kidney regeneration and function in some models of acute kidney injury (AKI), such as ischemia-reperfusion injury [107,108] or nephrectomy [109]. An increase in epithelial tubular cell proliferation and viability has been described [80,110], as well as a decrease in apoptosis, oxidative stress, and inflammation [80]. All these factors improved the recovery of renal function in these models. Other groups have studied the effect not only in AKI but also in chronic kidney damage. Stem cell-derived EVs have been shown to preserve renal functioning, improving cell survival through inhibition of profibrotic genes and apoptosis, showing a 30% decrease in BUN (blood urea nitrogen) in a mouse model of cyclosporin damage [111].

### 3.6. Effects of EVs on Damaged Liver

The adult liver is one of the tissues that are typically able to restore organ function after damage. Hepatocytes are active proliferating cells that can repopulate the tissue completely. However, hepatic failure can occur if the regenerative process is not adequate. EVs from different cells have been shown to have protective effects on liver damage, showing a decrease of around 40% in serum transaminases levels. EVs promote hepatocyte proliferation in drug-induced damage [112] and ischemia-reperfusion [81,113], decreasing apoptosis, oxidative stress, and excessive inflammation [114]. Similarly to other tissues, EVs decrease fibrosis progression in models of hepatic fibrosis [115].

## 4. Therapeutic Potential of EVs in Age-Related Diseases

As we have explained, EVs from different sources have an important role in several cellular and molecular processes associated with aging, even reversing some of these markers both in vivo and in vitro. Hence, it is fair to propose that EVs may have a therapeutic role in age-related diseases. The role of EVs in the pathophysiology of these diseases, along with their potential use as biomarkers for these, has been reviewed before [116–118]. Here, we will focus on some of the most relevant studies using EVs as a therapeutic tool in age-related conditions. A summary of the results described is shown in Figure 2.



**Figure 2.** Recapitulation of the effects of treatment with EVs in preclinical models of age-associated diseases. For each condition, observed effects of EVs on different biological processes are shown, with an arrow indicating the increase or decrease in a concrete process. BMSCs: bone marrow-derived stem cells; SASP: senescence-associated secretory phenotype.

#### 4.1. Alzheimer's Disease

EVs from healthy cells, mainly stem cells, are now being studied as potential therapeutics in AD. Using transgenic mice that develop Alzheimer's, multiple studies have shown that EVs from neural and mesenchymal stem cells can rescue cognitive decline associated

with AD [119,120]. EVs reduce several markers of the disease, such as amyloid- $\beta$  accumulation [120], oxidative damage [121], neuroinflammation, and microglia activation [122], along with an increase in dendritic spines. These studies have offered some candidate factors included in EVs that could be responsible for the effect, mainly miRNAs that are upregulated in EVs from stem cells [123].

#### 4.2. Atherosclerosis

Atherosclerosis development in different parts of the vasculature is a common feature of aging, and it has a major effect on the decline of tissue function associated with aging, being one of the most important contributors to cardiovascular disease [124]. Many drugs target the accumulation of lipids and slow the progression of the disease; however, we lack a disease-modifying treatment for atherosclerosis [125]. EVs from different cell types, such as MSCs [126] or platelets [127], have shown promising effects on the treatment of atherosclerosis and plaque development. These EVs have been shown to influence the behavior of cells associated with atherosclerosis; for example, MSCs-derived EVs can reduce macrophage infiltration through miRNA delivery [128], with anti-inflammatory effects on eosinophils and endothelial cells (ECs) [129,130]. In another study, the authors demonstrated that EVs and associated miRNAs from endothelial progenitor cells can target ECs in atherosclerotic plaques, reducing oxidative stress, inflammation, and endothelial contractile dysfunction [131].

#### 4.3. Type 2 Diabetes

Another relevant disease associated with aging and a contributor to the development of many age-related conditions is type 2 diabetes (T2DM) [132]. Altered glucose and other macromolecule metabolism is key in the pathogenesis of diabetes, something that is also altered with aging [62]. Researchers have studied the effect of EV treatment on some preclinical models of the disease. EVs from different sources, mainly MSCs, have shown therapeutic potential in T2DM [133]. The main effects are improved glucose and lipid metabolism, induction of autophagy [134], and inhibition of inflammatory response [135], all leading to improved insulin sensitivity. EVs from other sources, such as endothelial progenitor cells [136] or pancreatic  $\beta$  cells [137], have been shown to induce angiogenesis in pancreatic islets, promoting the survival of  $\beta$  cells and their function.

#### 4.4. Osteoporosis

Osteoporosis incidence rises as we age and is particularly abundant in the post-menopausal female population [138]. The main model used in mice to study osteoporosis is the induction of post-menopausal osteoporosis through ovariectomy in female mice. This model has been used to test the effect of EVs from different sources in the development of osteoporosis. Due to its close association with the bone, bone marrow-derived stem cells (BMSCs) are the most studied source of EVs for osteoporosis treatment; other interesting sources are serum and milk. EVs from these sources have shown a beneficial effect on the progression of osteoporosis [139,140]. The proposed mechanisms are the inhibition of osteoclasts resorption activity [141], an induction of osteogenesis through increased osteoblasts proliferation and activity [139,142,143], promotion of the osteogenic differentiation of BMSCs [143], and an increase in vascularization via angiogenesis [144]. Some proteins that regulate osteogenesis, such as CLEC11A or MALAT1, are present in EVs and have been proposed as potential factors implicated in the beneficial effect [140,145]. Other proposed factors are miRNAs included in EVs that can modulate different pathways associated with osteoporosis development [142,144].

#### 4.5. Osteoarthritis

One of the most common causes of disability in developed countries is osteoarthritis (OA). Nearly every person develops some degree of OA throughout their lives, with few therapeutic options apart from orthopedic treatment and pain management [146,147]. The

main risk factor for OA is aging, so targeting this process is now an approach to modifying the natural history of OA [146]. SCs are the main source of EVs studied for the treatment of OA, as intra-articular and systemic injections of different SCs populations have been an attractive field in regenerative medicine due to their immunomodulatory properties that reduce excessive inflammation in OA [148]. EVs from MSCs have demonstrated their potential as therapeutics in preclinical models of the disease, mainly surgical models and intra-articular injection of different toxins [149]. They have shown beneficial effects on extracellular matrix regeneration [150], promotion of chondrocyte survival [149], as well as immunomodulatory effects [151]. Some proteins [152], miRNAs [153,154], and even glycans [155] that are present in MSCs-derived EVs are proposed molecules with therapeutic effects by themselves and are now being studied as therapeutics for OA.

#### 4.6. Chronic Kidney Disease

Aging is usually accompanied by a progressive decline in renal function, and the incidence of CKD and renal failure rises as people age [156]. As we have stated before, EVs have been widely studied in kidney regeneration, especially those coming from MSCs. There are preclinical models of CKD in which EVs have been studied, mainly toxic induction of CKD [157], remnant kidney model [109], unilateral ureter obstruction [158,159], and diabetes [160]. EVs from MSCs have exhibited effects on a large number of processes associated with the development of CKD, with an effect not only on these processes but in renal function determined by serum urea and creatinine. These effects are inhibition of fibrosis [157], decrease in lymphocyte infiltration and inflammatory markers [109], induction of autophagy [47], and angiogenesis [161].

#### 4.7. Frailty

Per definition, frailty is the physiological state characterized by an increase in the vulnerability to external aggressions because of a decrease in the physiological reserves of several systems [162]. Frailty prevalence increases dramatically with age and is one of the most accurate predictors of dependence and mortality in old people [163]. The search for therapies to improve the decline in physical performance and to prevent sarcopenia is particularly attractive in the aging field. In the last year, we and others have demonstrated an effect of EVs from different sources in the muscle of old mice. Sahu et al. showed that circulating EVs in the plasma of young mice were able to rejuvenate old muscle cells and improve the muscle regenerative capacity of old mice [75], leading to an increase in fiber size, muscle force, and mitochondriogenesis, with a decrease in fibrosis. They proposed that Klotho transcripts (which decline with age) present in young EVs were the main factors responsible for the effects observed. More recently, we have evidenced a beneficial effect of EVs from young adipose-derived MSCs in the physical performance of old mice, as well as a decrease in frailty, these effects were accompanied by an increase in fiber size and muscle protein content, with a decrease in muscle senescence and SASP factors, oxidative stress and lipid deposition [24].

### 5. Overview

Aging is the most important factor for the development of many diseases that affect us, which are the main cause of disability and mortality in almost every country [164]. The use of therapeutics that target the causes of aging at a cellular and molecular level is one of the most promising fields of study in medicine [13,165]. We will probably need to target several of these processes to treat age-related diseases. In this sense, EVs have been demonstrated to regulate a wide range of cellular processes due to their capacity to mediate intercellular communication [8,166].

As we have exposed in this work, EVs from different sources, particularly those released by stem cells, due to their pro-regenerative and immunomodulatory properties, are presented as a cell-free therapy that has demonstrated substantial effects in preclinical models of several age-related diseases and tissue regeneration. EVs not only have beneficial

effects on diseases, but they have also been shown to regulate many processes associated with aging, with remarkable effects on the regenerative capacity of the tissues. In fact, in the last years, EVs from young mice and healthy cells have proven to recapitulate some of the effects of already proven interventions in aging, such as parabiosis, dietary restriction, mTOR inhibition, or senolytics [23–25]. Although these processes seem to be partly regulated by EVs, the different sources of EVs are also subjected to the aging process. As we have shown, the age of the donor is key to the beneficial effect of EVs [24]. The content in EVs differs between young and old cells. As cells become old, they start to release factors to the extracellular environment that result in deleterious effects such as paracrine senescence [19,20]. Thus, when developing therapeutics for age-related conditions, the use of young cells or plasma would probably bring better results. Regarding this, we still do not know the optimal source of EVs. Adult stem cells (mainly MSCs) seem to be a reasonable source for all the aforementioned properties. However, future studies should elucidate if other types of SCs, such as embryonic SCs or induced pluripotent SCs, have a greater potential in targeting the aging process.

The use of EVs as therapy by themselves is now being studied in clinics, with some clinical trials ongoing [167]. However, we still need to keep studying the basic biology of EVs and improving isolation and manufacturing methods to arrive at real patients. It is clear that we still need standardization protocols for the production, isolation, and manufacturing of EVs, as well as a need to understand the mechanisms of action before we can arrive at a feasible use of EVs in clinics [9,168]. There are two more alternatives to the use of whole EVs. The first is to use EVs as drug delivery vehicles, adding an interesting feature to the beneficial effect of EVs, with some studies showing the superiority of EVs loaded with different drugs or factors compared with EVs alone [169].

The second avenue of research is to explore EVs content and find different molecules or combinations of molecules that can recapitulate the beneficial effects observed with EVs treatments. This approach would probably result in a faster and simpler way for the clinics. In this regard, a great number of proteins, miRNAs, and lipids have been proposed in different studies. In preclinical studies, miRNAs are probably the most studied factors, as they can regulate many different pathways; however, some studies suggest that miRNAs are minor constituents of EVs with very low capacity to enter target cells [170,171].

Therefore, there is still a need for studies on the basic biology of EVs and the mechanisms that mediate both the release from donor cells and the effects on recipient cells. Nevertheless, we believe that both EVs and their content are interesting therapeutic tools in aging and age-related diseases, thanks to their ability to regulate many features associated with the aging process.

**Author Contributions:** J.S.-R.: Conceptualization, figure preparation, writing—original draft. C.M.-B., N.R.-G., J.H.-A. and M.D.: Conceptualization and writing—original draft. C.B.: Conceptualization, writing—reviewing and editing, supervision, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the following grants: Grant PID2020–113839RB-I00 funded by MCIN/AEI/10.13039/501100011033, PCIN-2017–117 of the Ministry of Economy and Competitiveness, and the EU Joint Programming Initiative ‘A Healthy Diet for a Healthy Life’ (JPI HDHL INTIMIC-085) to C.B., and CIGE/2021/134 from Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital to C.M.-B. Part of the equipment employed in this work has been funded by Generalitat Valenciana and co-financed with ERDF funds (OP ERDF of Comunitat Valenciana 2014–2020).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** We are sorry that some relevant work could not be cited due to space limitations. Figures were mounted using BioRender.

**Conflicts of Interest:** The authors declare no conflict of interest.

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