Chapter

BP-EVs: A Novel Source of EVs in the Nanocarrier Field

Cristina Lorca, María Fernández-Rhodes, Jose Antonio Sánchez Milán, María Mulet, Julia Lisa, Xavier Gallart-Palau and Aida Serra

Abstract

Extracellular vesicles (EVs) represent a complex mechanism of molecular exchange that has garnered significant attention in recent times. Nonetheless, identifying sustainable sources of biologically safe EVs remains challenging. This chapter delves into the utilization of fermented food industry by-products as a circular and secure reservoir of biocompatible EVs, dubbed as BP-EVs. BP-EVs demonstrate excellent oral bioavailability and biodistribution, with negligible cytotoxicity, and a preferential targeting capacity toward the central nervous system, liver, and skeletal tissues. The ease of editing BP-EVs is also depicted using the most common EV editing methods in this chapter. Globally, these groundbreaking findings are poised to unlock significant avenues for leveraging BP-EVs as an optimal source of biocompatible nanovesicles across a wide array of applications within the bioeconomy and biomedical fields. These applications primarily target molecule delivery into the central nervous system and skeletal tissue but are not limited to these two organism systems.

Keywords: extracellular vesicles, circular economy, nanocarriers, upcycling, compound delivery

1. Introduction

Extracellular vesicles (EVs) represent a sophisticated method of intercellular communication, characterized by resilient lipid membranes, specific molecular contents, and embedded signaling molecules. This mechanism has recently garnered tremendous interest [1]. These spherical vesicles are released and assimilated by almost all cell types across all domains of life [2]. Thanks to these unique attributes, EVs have been suggested to play crucial roles in both health and disease [3]. Furthermore, they have gained recognition as promising carriers for delivering drugs and bioactive compounds, sparking growing interest in their potential applications as nanocarriers [4].

While EVs possess favorable characteristics as nanocarriers—such as low immunogenicity, the capability to cross biological barriers, stable circulation, and organ targeting—there are significant challenges impeding their optimal use [4]. Issues related

to safety, scalability, and the identification of compatible physicochemical attributes present limitations [5]. These challenges arise partly because the primary sources of nanocarrier EVs in research are immortalized cell lines, which raise concerns about human safety and resource availability [6]. Similarly, the progress of EV mimetics, involving the laboratory-based creation of artificial EVs and liposomes as potential nanocarriers, is hindered by the limitations of current EV sources [7].

Here, we present food industry by-products derived EVs (BP-EVs) (European Patent PCT/EP2022/080507) [8], a novel nanocarriers platform based on the use of EVs obtained from fermented food industry by-products (FFBP). FFBP represents a safe, circular economy friendly, and inexpensive source of EVs. BP-EVs exhibit no cytotoxicity, as they highly resemble the daily consumed food-derived EVs, and display excellent oral and intravenous bioavailability as well as specific organ targeting capacity, with preferential targeting capacity toward the central nervous system (CNS), skeletal tissue, and liver. Additionally, BP-EVs can be easily edited by different methods. Collectively, we believe that BP-EVs will open substantial venues as an optimal source of biocompatible nanovesicles in manifold applications of the bioeconomy and biomedical fields.

2. Extracellular vesicles

Extracellular vesicles (EVs) are a heterogeneous group of spherical nanoparticles ranging in size from 30 to 5000 nm, naturally produced by cells, and delimited by a phospholipid (PL) bilayer membrane [9]. These rounded structures, which cannot replicate themselves, consist of lipids, proteins, and nucleic acids, and function as tiny vehicles for transporting, protecting, and delivering a wide diversity of cargoes [9]. Despite the crucial functions performed by these vesicles and their excellent properties as biomarkers for human diseases, EVs were long considered mere residues, often referred to as "cellular dust," and were overlooked for decades [10]. Nowadays, we know that EVs are produced by most, if not all, cell types and can be found in all biological fluids. According to the International Society for Extracellular Vesicles (ISEV), the term "EVs" should be reserved for those particles enclosed by a cellular membrane, naturally released by cells, and incapable of replication. Based on the ISEV's classification, EVs are further categorized into exosomes, microvesicles, and apoptotic bodies, depending on their biogenesis pathway [11] as illustrated in **Figure 1**.

EVs have been confirmed as a potent communication system between both same and different organisms, capable of serving as vehicles for signaling molecules between cells at close and distant locations, thus functioning as mediators at all four levels of communication (autocrine, direct, paracrine and endocrine) [9]. In contrast to other signaling particles, such as hormones, EVs not only have the ability to simultaneously package and protect a variety of messenger molecules, including hydrophilic and hydrophobic substances, but they also have complex compositions that provide direct information from the progenitor cell [12]. Additionally, it has been confirmed that some of these vesicles possess the ability to preferentially deliver their contents to specific cell types or tissues [13]. This groundbreaking discovery has opened up an entirely unexplored and multidisciplinary research field, offering promising potential for using these particles as tools to better understand and eventually intervene in cellular cross-talk. Furthermore, recent evidence points to EVs as the particles with the highest potential for use as editable and specific-target nanocarriers in medicine in the near future [13].

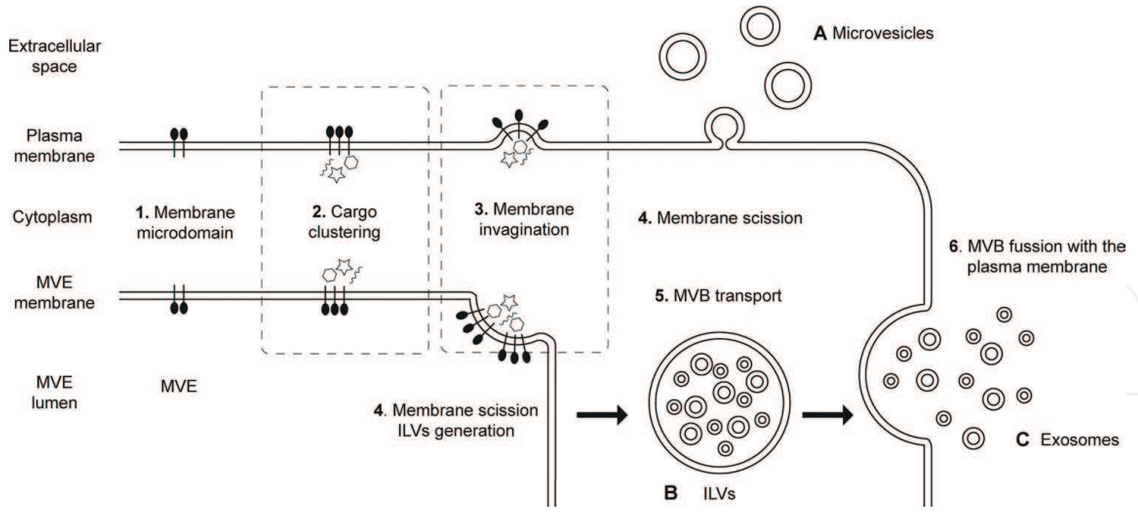

Figure 1.
EVs biogenesis pathways. 1. Potential EV components and cargoes are dispersed throughout the cytosol and membranes. 2. Specific elements and cargoes that will constitute future EVs are clustered and recruited. 3. The recruited elements contribute to membrane bending and invagination. 4. Membrane scission leads to the generation of microvesicles (A) or intraluminal vesicles (B). 5. Transport of the multivesicular body (MVB) to the plasma membrane. 6. Fusion of the MVB with the plasma membrane and release of the intraluminal vesicles (now exosomes) into the extracellular space.

2.1 EVs from different life kingdoms

2.1.1 Fungal EVs

Although first observed in the early 1970s, fungal EVs (F-EVs) were not properly described until 2007 in the ubiquitous encapsulated yeast *Cryptococcus neoformans* [14]. Similar to bacterial and plant cells, fungal cells are protected by a thick cell wall composed of glycoproteins and complex carbohydrates, including the fungal-specific polysaccharide chitin [15]. Although the cell wall was initially thought to prevent vesicular transit due to its rigidity and absence of large pores, we now know that cell walls are flexible structures that can be easily rearranged for cellular division or during EV generation. In the last decades, it has been shown that F-EVs are fundamental for key biological functions, such as cell wall remodeling [16], biofilm matrix formation [17], and host-pathogen interactions [18]; and that these particles are clearly able to modify the behavior of recipient cells [19]. To date, F-EV production has been confirmed in several fungal species, including yeasts such as *Saccharomyces cerevisiae* [16], known for its role in beer production, or the opportunistic pathogen *Candida albicans* [20], which is also a common member of the human microbiota. F-EV production has also been proven in filamentous fungi such as *Aspergillus fumigatus* and *A. flavus* [21], which cause invasive aspergillosis.

F-EVs share a classification similar to that of mammalian EVs based on their biogenesis into exosome and microvesicle-like structures. While the full extent of EV production in fungi remains incompletely understood, there are similarities in pathways and genes with mammalian vesicles [22]. Notably, the production processes, such as the endosomal pathway, are highly evolutionarily conserved mechanisms. In addition to this, in fungi, periplasmic vesicles (PVs) have been observed between cells and their cell wall. However, it remains uncertain whether PVs are identical structures or fulfill similar roles to F-EVs found outside cells [23].

2.1.2 Plant EVs

While plant extracellular vesicles (P-EVs) were first observed in 1967, predating the discovery of mammalian EVs, they have received considerably less attention, and the pathways responsible for their generation remain largely unexplored. These pathways encompass various mechanisms, including fusion of multivesicular bodies (MVBs) with the plasma membrane, resulting in the production of exosomes, as well as budding from the plasma membrane, leading to the formation of microvesicles or apoptotic bodies. Additionally, there is the process of exocyst-positive organelle (EXPO)-mediated secretion, which generates EXPO-positive vesicles [24]. Furthermore, the mechanisms facilitating the passage of P-EVs through the cell wall to reach the extracellular space, along with the diverse secretion pathways, remain incompletely understood. Consequently, the classification of P-EVs is still in its early stages. P-EVs could potentially be categorized based on the presence of specific markers associated with the particular generation mechanism of P-EVs, giving rise to at least three subcategories [25]: TET-positive exosomes, characterized by the presence of TETRASPANIN (TET)-like proteins and originating from MVBs; EXPO-derived EVs, which originate from the EXPO organelle; and PEN1-positive EVs, composed of the penetration 1 protein, whose origin is as yet unknown but which appears to play a role in stress responses [25]. Similar to other biological kingdoms, P-EVs serve as pivotal regulators in essential processes, with their noteworthy contribution extending to cellular defense against pathogens [25].

2.1.3 Bacterial EVs

Bacterial extracellular vesicles (B-EVs) typically range from 20 to 400 nm in diameter and can be classified based on their structure, composition, and origin. The primary distinction lies in whether they are produced by Gram-negative or Gram-positive bacteria, as their differing structures are reflected in the vesicle's architecture [26].

2.1.3.1 Gram-negative bacteria-derived EVs

Gram-negative bacteria possess two double-layered membranes: the outer membrane, which is rich in lipopolysaccharide (LPS) on its outer leaflet, and the inner or cytoplasmic membrane, separated by a periplasmic space containing peptidoglycan [27]. Among the extracellular vesicles (EVs) produced by Gram-negative bacteria, outer membrane vesicles (OMVs) are predominant, typically ranging in size from 50 to 250 nm. As their name suggests, OMVs originate directly from the outer membrane, making them covered in LPS and enriched in outer membrane proteins. They also contain periplasmic components and exhibit specific lipid compositions [28]. Additionally, OMVs may contain cytoplasmic molecules, although the presence and sorting mechanisms for cargo selection have not been fully elucidated [27]. In contrast to conventional EVs, outer-inner membrane vesicles are encased by not one but two bilayer lipid membranes, corresponding to the outer and inner membranes of Gram-negative bacteria. Similar to OMVs, these particles feature a rich outer membrane with LPS and contain peptidoglycan in the periplasmic space [26].

2.1.3.2 Gram-positive bacteria-derived EVs

In contrast to Gram-negative bacteria, Gram-positive bacteria have a plasma membrane covered by a thick layer of peptidoglycan, which must be crossed by secreted

vesicles to reach the extracellular space [29]. The term "cytoplasmic membrane vesicles" (CMVs) is used to describe B-EVs generated by Gram-positive bacteria. Since these B-EVs lack an outer membrane, B-EVs originating from dying cells are also considered CMVs. In both cases, the production of CMVs is triggered by endolysin, enabling these vesicles to bud into the extracellular space, crossing the peptidoglycan barrier [26]. Established functions for B-EVs include the transmission of virulence factors, nucleic acids, and defense factors to hosts, antibiotics, or bacteriophages [26]. Additionally, B-EVs play an intriguing role in ecosystems, such as their participation in the carbon cycle in marine environments [30].

3. EVs as nanotransporters

The efficacy of drug delivery faces constraints due to the instability of drugs within the body and their inability to reach the target tissue. This often necessitates the use of carriers for efficient drug delivery. Drug delivery systems encompass technologies that package and transport drugs within the body, overcoming pharmacokinetic challenges and natural bodily limitations to enhance their effectiveness [4]. In the quest for effective vectors for targeted drug delivery, various approaches have been explored in recent decades. A diverse range of synthetic vehicles, including liposomes, microspheres, and polymeric nanoparticles, has been employed to distribute drugs throughout the body. These systems can transport various types of drugs, including small molecules, proteins, nucleic acids, and antibodies [31]. However, a drawback of these synthetic carriers is their tendency to elicit a toxic immune response when recognized as foreign particles.

Extracellular vesicles (EVs) have emerged as promising nanotransporters in medicine due to their ability to deliver various bioactive molecules, such as proteins, lipids, and nucleic acids, to specific cells and tissues. EVs possess unique properties and offer appealing advantages over synthetic nanocarriers, including superior cellular uptake, high stability, biocompatibility, low immunogenicity, the capability to cross biological barriers, such as the BBB, cargo protection, and the potential for targeted delivery of bioactive molecules [32]. When used as drug nanocarriers, EVs can significantly enhance pharmacological efficacy while reducing drug toxicity. Other potential applications of EVs encompass gene therapy, immunotherapy, vaccine development, and tissue engineering [33]. For example, they can be utilized to deliver bioactive molecules that promote tissue repair [34]. Consequently, developing proper strategies for employing EVs as nanocarriers would enable their application in a wide range of medical scenarios [35].

3.1 Endogenous EVs advantages over synthetic nanocarriers

Synthetic nanoparticles have been extensively studied for their potential as carriers [36]. In general, synthetic nanoparticles are much simpler in structure compared to EVs, offering appealing characteristics such as ease of mass production in a cost-effective and time-efficient manner, known composition, and simpler standardization protocols [37]. However, when applied to living organisms, these engineered particles present inherent challenges that have hindered their full realization as efficient nanocarriers [36]. One of the primary concerns lies in their lack of biocompatibility and potential toxicity. Engineered materials can trigger immune responses and cause cytotoxicity, limiting their application in biomedical settings [38]. Moreover,

synthetic nanoparticles often encounter accumulation issues, which can lead to potential long-term adverse effects [39]. Additionally, challenges arise regarding the cellular delivery capacity of synthetic carriers and their ability to traverse physiological barriers such as the blood-brain barrier (BBB), which restricts their access to vital target tissues, such as the brain [40].

In contrast, native EVs, which are naturally produced by cells, possess inherent biocompatibility, leading to reduced immunogenicity [41] and fewer toxicity concerns. As endogenous carriers, EVs are readily recognized by recipient cells and exhibit longer retention times in circulation compared to synthetic counterparts [42]. Their natural ability to interact with specific cell types facilitates targeted delivery [43], minimizing off-target effects and enhancing therapeutic efficacy. Furthermore, EVs have shown remarkable potential in crossing physiological barriers, including the BBB [44], which facilitates the delivery of cargo to the CNS for targeted brain therapies. Notably, this attribute makes them particularly attractive for treating neurological disorders and brain-related diseases. Nonetheless, EVs are not without challenges. Obtaining EVs in sufficient quantities remains one of the major obstacles [45]. Safety is another concern, as EVs derived from cancerous cells have been shown to be tumorigenic, raising concerns when choosing EVs derived from immortalized cell cultures, which currently represent one of the main sources [46]. Additionally, standardizing isolation protocols can be intricate due to the high heterogeneity of these particles [45]. Although naturally produced EVs emerge as a superior choice for biomedical nanocarriers, outperforming synthetic nanoparticles in terms of biocompatibility, targeted delivery, and overcoming physiological barriers, there are still critical challenges that need to be addressed to fully harness their biomedical potential [47].

3.2 Challenges to implement EVs as nanocarriers

Given the potential of EVs as nanocarriers, there is currently a significant and growing interest in researching EVs as pharmacological transporters for various substances, including chemotherapeutics [48]. However, numerous obstacles still exist that impede the clinical utilization of these particles [49]. The major challenges include obtaining a sufficient yield, isolating, storing, standardizing procedures, characterizing EVs, ensuring safety, loading them with cargo, and editing their targeting capabilities [50]. Currently, one of the most common sources for generating EVs for use as therapeutic nanocarriers is immortalized cell lines, including adherent stem or immune cells grown in 2D cultures [51]. However, this presents a significant limitation as cell cultures produce EVs in small quantities [52], and adapting these settings to grow in suspension can be nontrivial, hindering their transferability to the pharmaceutical and biotechnology industries. Another challenge arising from the use of cell lines as a source of EVs is safety concerns [53], especially when dealing with EVs derived from tumor cell lines that may have tumorigenic effects. To function as transporters, EVs need to be loaded with the desired cargo, and different strategies can be applied depending on the physicochemical properties of the cargo [53]. These various loading strategies will also be reviewed in this chapter. Finally, depending on the application, the EV surface may need to be modified to achieve the desired biodistribution and targeting properties while preserving other relevant traits, such as low immune recognition and stability [54]. Despite these challenges, the interest and potential of these vesicles are evident, reflected not only in the increasing academic research each year but also in the growing number of companies offering products for EV isolation, purification, characterization, and engineering, as well as conducting preclinical and clinical trials [55].

4. BP-EVs: a novel approach for safe and accessible nanocarriers

BP-EVs, an asset protected under patent (PCT/EP2022/080507) since November 2021, are EVs enriched from FFBP derived from the production or processing of animal-based foods, such as kefir and other fermented dairy items, as well as plant-based foods like beer and wine, among others [8]. Generally, plant-based BP-derived BP-EVs are produced by yeast and bacteria, while dairy-based BP-derived BP-EVs are predominantly produced by mammalian EVs [8]. With diameters ranging from 30 to 950 nm, with 50% falling below 200 nm, signifying exosome enrichment, BP-EVs share similar lipidomic and proteomic attributes with food-derived EVs, demonstrating no cytotoxicity [8]. BP-EVs exhibit remarkable oral bioavailability, showing no disparity compared to intravenous administration, and exceptional biodistribution. Notably, these vesicles are rich in exosome markers and demonstrate distinctive *in vivo* targeting, particularly toward the CNS, liver, and skeletal tissues. The most efficient method for obtaining BP-EVs, as elaborated in the patent, involves an initial centrifugation phase to separate cells and insoluble debris from the BP solution, followed by a sequence of washing and filtration steps to eliminate soluble components from the source material and concentrate the vesicles [8].

When considering the challenges of utilizing EVs as nanocarriers, particularly regarding the acquisition of these particles in sufficient quantities, BP-EVs have successfully demonstrated that FFBPs provide a practical and cost-effective alternative for obtaining safe and biocompatible EVs. These EVs have the potential to be employed as targeted nanotransporters [8]. The industrially scalable strategy, coupled with the sister industrial technology of tangential filtration, for obtaining EVs from FFBPs offers an innovative and practical solution to overcome some of the major challenges in EV research and exploitation as nanocarriers [8].

4.1 BP-EVs: transforming industrial waste into a valuable asset for human health

Waste from food production is generated in large quantities, posing significant environmental problems and resulting in substantial handling expenses. During the development of BP-EVs, we embraced circular economy principles in line with the Sustainable Development Goals of the European Union. By harnessing FFBPs as a sustainable source for safe and biocompatible EVs, we created an innovative, upcycling, and environmentally conscious strategy. This strategy transforms otherwise discarded food industry waste into advanced nanocarriers, representing a valuable biomedical resource that addresses industrial waste challenges while unlocking research opportunities for EVs in biomedical applications. Altogether, BP-EVs are positioned as treasured assets for their potential as nanocarriers.

4.2 Drug delivery and potential applications of BP-EVs

BP-EVs possess a notable advantage due to their inherent low immunogenicity and cytotoxicity, which stems from their biological origin. Additionally, BP-EVs have demonstrated the capability to traverse biological barriers, potentially opening new avenues for treatments targeting organs that have historically been challenging to access, such as the brain [8]. The primary potential market for the application of BP-EVs lies in human health within the bioeconomy and biomedical fields. It is worth noting that the scope of potential applications of BP-EVs extends beyond the biomedical field. These versatile particles have the potential to be used in other industries,

impacting various sectors, including cosmetics and nutrition, among others. While researchers continue to unravel the intricate mechanisms underlying the physiology and behavior of these remarkable particles, these endeavors bring significant hope for the early application of these vesicles as targeted nanocarriers, thereby improving the effectiveness of multiple therapeutic interventions.

Given their natural capacity to reach the brain, BP-EVs could facilitate passage through the BBB and effectively transport drugs to target the CNS. It is worth noting that the market for CNS-focused treatments represents a global market estimated at $612 million in 2022, and it is expected to grow at a compound annual growth rate (CAGR) of 8.9%, reaching $938 million by 2027. An example of a potential application would be the treatment of glioblastoma multiforme, the most common and deadliest form of brain cancer in adults, which currently has a 5-year relative survival rate of only 5% [56]. However, the treatment of other CNS diseases, such as neurodegenerative diseases, psychiatric disorders, and various types of CNS cancers, could also be substantially improved through the use of these advanced nanocarriers.

Additionally, given their ability to preferentially accumulate in bone tissue, it is plausible to employ BP-EVs for delivering therapeutics specifically to the bone. This is particularly relevant because bone is a tissue that can be difficult to reach due to the avascular cartilage, often necessitating high doses for effective treatment, which can lead to elevated off-target toxicity [57]. An illustrative example of a potential application is in the treatment of osteoporosis, a metabolic disorder that compromises bone strength, resulting in an increased risk of fractures and contributing to morbidity and mortality for patients [58]. Notably, the market for osteoporosis treatment was valued at $14 billion in 2022, with a CAGR of 3.8%. It is important to note that the liver targeting capacity of BP-EVs is not discussed in this section, as the presence of vesicles in this organ may be associated in part with detoxification and excretion [59]. Further research is needed to investigate the potential use of BP-EVs for targeting the liver in the treatment of liver diseases.

5. Editing methods

There are different editing methods that enable the engineering of BP-EVs and EVs in general to serve as nanocarriers, allowing the modification of their properties to achieve desired therapeutic effects. EVs can be tailored to carry molecules of interest either internally or externally, to reduce their clearance by natural systems (such as the immune, hepatic, and renal systems), to exhibit tropism to specific microenvironments (e.g., low pH), to target specific cell types or tissues, to enhance their intracellular cargo delivery capabilities, or to activate cells in specific ways, among other objectives. Editing methods can be categorized based on their objectives, which may involve modifying molecules transported by EVs or influencing EV targeting and interactions within their environment. This section provides a detailed overview of the most common methods for editing EVs.

5.1 Cargo loading

5.1.1 Passive cargo loading

The simplest and most convenient technique for cargo loading involves incubating desired cargoes with EVs. This method relies on a passive transport mechanism that takes advantage of the concentration gradient, allowing for passive diffusion.

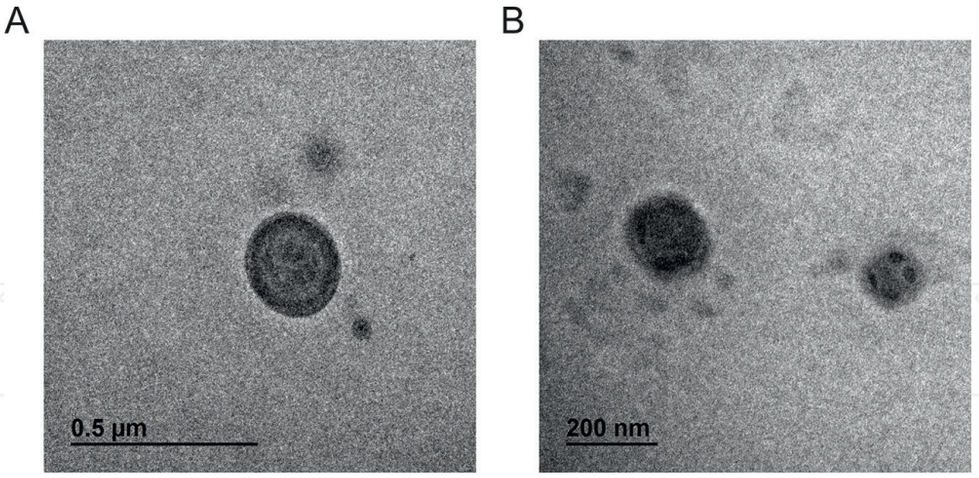

Figure 2.
Transmission electron microscopy images of a. unloaded BP-EVs and B. BP-EVs loaded by passive diffusion with the plant extract BacA from the nootropic plant Bacopa monieri. Ultrastructure of BP-EVs was not altered during the loading process.

This facilitates the spontaneous incorporation of hydrophobic cargos into EVs or EV-secreting cells [60]. The loading efficiency of this strategy is generally low and influenced by the polarity of the cargo [61]. Other factors influencing the process include temperature, incubation time [35], pH, and cargo concentration [54]. Passive cargo loading typically does not affect the ultrastructural properties of EVs, as demonstrated in **Figure 2**.

Within passive cargo loading strategies, the pH gradient method is based on the fact that exosomes typically have an internal pH of 9. This method creates a pH gradient between the inside (at pH 9) and outside of the EVs (ideally at pH 4.5). The generated pH gradient can increase loading efficiency by up to three times [62].

Another variant of passive cargo loading is hypotonic dialysis, which involves mixing cargo and EVs in a dialysis membrane or tube to obtain cargo-loaded EVs as a result of the differential concentration gradient [63]. This method has been reported to increase loading efficiency by more than 11-fold compared to incubation [64].

5.1.2 Active cargo loading

5.1.2.1 Physical methods

In the freeze-thaw method, the mixture of EVs and cargoes is exposed to several cycles (typically from 5 to 10) of freezing temperatures below −70°C, followed by rapid thawing at room temperature [65]. This temperature shift disrupts membranes, allowing for the loading of small molecules. This simple technique can cause EVs to fuse and has also been successfully used to merge EVs with liposomes, creating EV-mimetic particles [66]. However, it has a moderate encapsulation efficiency, lower than that of other physical techniques such as electroporation or sonication [49].

Electroporation uses short and high-voltage electrical pulses to temporarily create small holes in membranes, increasing their permeability and allowing hydrophilic cargoes to diffuse into EVs [67]. This technique is one of the most commonly used for EV loading. The applied potential can vary significantly, ranging from 0.1 to 1000 kV, depending on the specific case. This method offers good loading efficiency and is easy to operate [55]. However, electroporation can potentially affect membrane integrity [67].

Sonication applies sound waves to generate transient pores or even break down and reconstitute EVs [68], allowing for the passive diffusion of cargo molecules within the membrane. This method offers high loading efficiency [69]; however, it may compromise the structural integrity of EVs and generate smaller particles compared to other techniques [70].

Extrusion involves forcing the vesicles through small pores, which can result in mechanical destruction and reformation of EVs. It employs an extruder device equipped with a heating block and polycarbonate filters with specific pore sizes, typically ranging from 100 to 400 nm. The cargo can enter the EVs by repeatedly pushing the mixture of vesicles and cargo through the filters [71]. This method provides relatively high packing efficiency and a uniform EV size distribution [72]. However, this process can damage the vesicles, including their membranes (which can alter their zeta potential) and proteins [64].

5.1.2.2 Chemical methods

Surfactant treatment involves the use of a reagent such as saponin or Triton, which creates pores in the membranes of EVs or cells, increasing their permeability. This allows cargo to enter more easily and significantly enhances the loading rate [73].

Transfection employs a specific vector to facilitate cargo loading into EVs. Among the agents used are reactives like calcium phosphate [74], diethylaminoethyl-dextran [75], polyethyleneimine [76], or cell-penetrating peptides [77]. Structures such as liposomes can also be employed to introduce larger cargoes, such as the CRISPR/Cas9 system, through merging with EVs [78]. It is important to note that some vectors may potentially damage EVs, cells, or cargoes [62].

5.2 Surface editing

5.2.1 Chemical modification of EV surface

There are different surface engineering strategies available to enhance the ability of EVs to successfully deliver their cargo to specific destinations. Chemical modification techniques use either covalent or non-covalent interactions to bind specific molecules to EV membranes without disrupting them [79]. Through covalent binding, various functional molecules such as small peptides, proteins, or polymers can be strongly attached to EV surfaces [49]. However, these techniques may require toxic chemicals and should be used with caution for editing therapeutic EVs [80]. Additionally, they often necessitate further purification steps [81]. Non-covalent binding can also be employed to modify EV membranes in a stable manner [82]. Another strategy for non-covalent binding is based on multivalent electrostatic interactions, which allow for the coating of EVs with a positive charge. This enhances their ability to target biological membranes, which typically have a negative charge, and facilitates their uptake by cells [83]. It is important to note that cationic nanomaterials can potentially cause cytotoxicity by disrupting membranes [84].

5.2.2 Other methods for the modification of EV surface

Other strategies for EV surface editing include hybridization with liposomes, resulting in the generation of larger, mimetic particles with mixed surface

properties. This technique has been used not only to incorporate larger cargos into mimetic-EVs [85], but also to enhance stability, prolong retention time, and improve cellular uptake [80].

6. Conclusion

The extensive details provided in this chapter position BP-EVs as ideal candidates for the next generation of nanocarriers in the field of biotechnology and biomedicine. Their potential applications are manifold, but primarily focused on the delivery of drugs into the CNS or poorly vascularized skeletal tissue. Thus, their impact on current and future developments in the field is poised to be transformative.

EVs have shown remarkable potential as advanced nanocarriers for delivering substances with a wide range of applications in the fields of biotechnology and biomedicine. BP-EVs exhibit a predominantly exosomal nature and possess exceptional biocompatibility. They also demonstrate the ability to traverse biological barriers and offer excellent oral bioavailability. BP-EVs can be easily edited by physical methods, although other strategies can also be applied to extensively modify their external and internal composition.

While BP-EVs offer numerous advantages as potential nanocarriers for drug delivery and other applications, they also have some drawbacks and challenges. Although BP-EVs are generally considered safe and biocompatible, safety concerns can arise if they are derived from specific food by-products that may contain allergens (i.e., BP-EVs derived from dairy FFBP). Thorough safety assessments are necessary to ensure their suitability for medical applications. Regarding particles diversity, like other types of EVs, BP-EVs can exhibit heterogeneity in terms of size, cargo content, and surface properties. This heterogeneity can complicate their characterization and standardization for specific applications. Finally, it is important to consider that like any novel therapeutic or drug delivery system, BP-EVs may face regulatory hurdles and require extensive testing and approvals before they can be used in clinical applications.

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Conflict of interest

The authors declare no conflict of interest.

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