Plant-Derived Extracellular Vesicles for Next-Generation Drug Delivery

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Plant cells release tiny membranous vesicles called extracellular vesicles (EVs), which are rich in lipids, proteins, nucleic acids, and pharmacologically active compounds. These plant-derived EVs (PDEVs) are safe and easily extractable and have been shown to have therapeutic effects against inflammation, cancer, bacteria, and aging. They have shown promise in preventing or treating colitis, cancer, alcoholic liver disease, and even COVID-19. PDEVs can also be used as natural carriers for small-molecule drugs and nucleic acids through various administration routes such as oral, transdermal, or injection. The unique advantages of PDEVs make them highly competitive in clinical applications and preventive healthcare products in the future.

plant-derived extracellular vesicles | next-generation drug delivery | toxicology | exosomes | drug delivery

1. Introduction

Extracellular vesicles (EVs) are unique lipid-bound membrane vesicles secreted in both plant and animal cells that enclose unique and powerful biochemically active compounds such as miRNA, lipids, proteins, and small biomolecules generated through the multivesicular body pathway or from the plasma membrane to the extracellular space [1]. Their size from animal origin is typically divided into four main categories of extracellular vesicles that result from different processes of formation, and they can be roughly differentiated based on their size. These categories are exosomes (50–150 nm), microvesicles (100–1000 nm), large oncosomes (1000–10,000 nm), and apoptotic bodies (100–5000 nm) [2]. However, recently, researchers have been able to isolate and characterize EVs of plant origin that are typically in the range of 30–500 nm [3][4], with some EVs isolated from carrots being as large as 1500 nm [5].

The differences between plant- and animal-derived EVs do not end there, as there are clear and startling differences. PDEVs were found to have natural anticancer, anti-tumor, antioxidant, anti-inflammatory, and wound-healing effects, among many others, from a seemingly limitless plethora of fruits and vegetables, each with their own unique and powerful therapeutic effects. It is noteworthy that ginger- and grapefruit-derived EVs have been studied extensively [6]. These innate natural therapeutic properties can be directly translated to human health, as not only are these effects beneficial, but there have been no reported adverse effects [7].

Interestingly, they were also found to play an important role in inter-kingdom communication that contributes to the symbiotic relationship between plants and micro-organisms and a host of other mechanisms [8][9]. In addition, compared to chemically synthesized article nanocarriers, plant EVs are not only safe, non-immunogenic, and non-toxic [10] but, fascinatingly, they can pass through the blood-brain barrier, while being able to deliver drugs to the pregnant mother without affecting the fetus [10], making it a game-changing avenue for the future of drug design.

Limitations of chemically synthesized nanocarriers and animal-derived EVs in drug delivery include, but are not limited to, low drug loading efficiency, low yield, short bioavailability due to short half-life in circulation, limited target tissue uptake efficiency, biocompatibility or immunogenicity issues, safety and ethical concerns (for EVs derived from animal sources, including human sources), and being unsustainable and unscalable for large-scale production [11][12][13].

Plant EVs by comparison can be scaled up economically, while being stable even within the gastrointestinal tract, carrying a variety of cargo loads, such as siRNA, miRNA, and even CRISPR/Cas9, with efficient absorption, and therefore, their commercial viability is much more promising than chemically synthesized or animal origin nanocarriers [14].

2. Composition of PDEVs

2.1. Structure

Structurally, animal- and plant-derived EVs are similar in nature. They are both membranous structures constructed of lipid bilayers, loaded with a variety of biochemically active compounds and substances such as nucleic acids, lipids, and proteins.

In terms of particle size, PDEVs have been found to be typically similar compared to animal EVs and range in size between 30 and 1500 nm, displaying a high level of stability, with a zeta potential that is generally negative and above the range of −20 mV [4][15][16][17][18].

2.2. Composition

PDEVs contain unique cargo loads depending on the type and source of plant or fruit, which in turn determines their biochemical functionality, natural innate therapeutic properties, bioavailability, and related processes.

The most important and well-studied of these exosomal cargo loads is miRNAs, which are short non-coding RNAs of an average size of about 22 nucleotides (nt), capable of regulating gene expression by specifically binding and inhibiting translation function or cleaving of target mRNAs [19]. Inter-kingdom communication and the functionality of PDEVs have been attributed to miRNAs. PDEVs have been shown to contain and deliver functionally active miRNAs from dietary sources and are capable of surviving the harsh environment of the digestive tract [20][21]. It was shown that not only are they able to survive and be absorbed but they are also capable of traveling to neighboring or distant regions of the body to regulate gene expression of humans, which may indicate a

pronounced effect on the role of dietary EVs of plant origin on the health and physiochemical function and regulation of human health and nutrition [22]. Several studies have shown that EVs derived from a plethora of plants of various species have been implicated to have miRNAs that target genes associated with several cancer- and inflammation-related pathways. Of note, ginger-derived miRNAs were found to inhibit lung inflammation caused by EVs released from SARS-CoV-2 [23].

As for studies on the protein content of PDEVs, it is severely limited due to the lack of proper databases of proteomic analysis, contaminations from cytosolic or enzyme proteins, and most importantly, their typically low protein concentration; however, it was seen that this can change significantly in response to biotic and abiotic stresses such as external infections by pathogens [24][25][26].

In contrast, lipids, specifically phospholipids, derived from PDEVs are well studied and known to play a vital role in plant exosomal stability and the uptake by specific targeting of cells, such as the gut and the liver, and their lipid composition affects which recipient cells are targeted and thereby affected by its cargo [27][28][29]. This is in contrast to lipids of animal origin, as they are predominantly sphingomyelin and cholesterol [1]. It has also been shown that nanocarriers derived from plant-origin exosomal lipids have been used with resounding success for the delivery of siRNA and anticancer agents [10].

It has been shown that PDEVs even carry secondary metabolites, such as flavonoids, chlorophylls, and curcuminoids, which were found to differ based on the origin of the plant/fruit species [10][26][27]. These secondary metabolites have been linked to their potential antimicrobial therapeutic activity and shown to increase in concentration seemingly in response to infections from pathogenic organisms [26][27].

3. PDEVs as Next-Generation Drug Carriers for the Treatment of Diseases

PDEVs, which are nanoparticles encased in lipid bilayers and contain various substances, can be used as drug delivery platforms. They can be loaded with small-molecule drugs or nucleic acid drugs without degradation, making them an effective method for preventing drug damage [30]. PDEVs have high bioavailability and can be absorbed in the intestine and penetrate deep into the skin [31]. They can pass through the blood-brain barrier and enter the brain when administered nasally [31], while being unable to pass from the mother to the fetus through the placenta [32].

PDEVs are also considered safe, as they have low toxicity and fewer side effects compared to synthetic lipid nanoparticles. The current method of drug delivery using PDEVs involves loading drugs onto the EVs or nanoparticles made from the extracted lipid components of the EVs. The majority of drugs used are nucleic acid drugs and small-molecule chemical drugs [33].

3.1. Capability of Delivering Nucleic Acids

PDEVs are capable of delivering nucleic acids, such as siRNA and miRNA. For example, grapefruit-derived EVs loaded with miR17 have been shown to be effective in treating brain tumors in mice by delaying their growth [34].

This is because EVs can reach the brain through nasal administration, allowing the miR17 they carry to also enter the brain and exhibit an inhibitory effect. Additionally, EVs combined with folic acid have been found to enhance the targeting of cytomegalovirus to folate receptor-positive brain tumors, providing a non-invasive treatment option for brain-related diseases [34].

There are multiple types of PDEVs that can be utilized for a range of delivery pathways. For example, acerola-derived EVs have been found to deliver small RNA to the digestive system, with the target gene-suppressing effect in the small intestine and liver peaking a day after administration, making it a potential option for oral delivery of nucleic acids [35]. Ginger-derived EVs loaded with siRNA-CD98 have also been found to effectively target colon tissue and reduce the expression of CD98 through oral administration [36].

Several methods can be used to introduce exogenous nucleic acids into plant-derived extracellular vesicles (PDEVs) [35][37][38][39][40]. Some of the common methods include:

- Electroporation: This involves applying an electric field to PDEVs and exogenous nucleic acids, causing transient pores to form in the membrane and allowing the nucleic acids to enter the PDEVs.
- Sonication: This method involves exposing PDEVs and nucleic acids to high-frequency sound waves, which disrupt the vesicle membrane and enable nucleic acids to enter the PDEVs.
- Incubation: PDEVs can be incubated with nucleic acids in a buffer solution under controlled conditions, such as temperature, pH, and salt concentration. This method allows nucleic acids to be passively taken up by the PDEVs.
- Extrusion: This involves forcing PDEVs and nucleic acids through a membrane with small pores, which mechanically disrupts the vesicle membrane and enables the nucleic acids to enter the PDEVs.
- Chemical transfection: This involves treating PDEVs and nucleic acids with chemicals that increase the permeability of the vesicle membrane, allowing the nucleic acids to enter the PDEVs.

It is worth noting that the efficacy and specificity of these methods may vary depending on the type of nucleic acid and PDEVs used, as well as the experimental conditions.

These studies demonstrate that PDEVs can serve as a drug delivery platform for small-molecule drugs, offering further potential applications.

3.2. Capability of Delivering Small Molecules and Drugs

PDEVs can be taken orally and have different cellular targeting due to their different sources [41]. They can also reach the brain via nasal delivery and are intercepted by the placental barrier, making them a highly effective drug delivery option. Grapefruit-derived EVs are capable of transporting drugs, such as chemotherapy agents, nucleic acids, and proteins, to various cells [32].

The targeting efficiency of cells that express folate receptors has been improved through the co-delivery of therapeutic agents and folic acid with *grapefruit-derived EVs*. The efficacy of *grapefruit-derived EVs* in inhibiting tumor growth through chemotherapy was demonstrated in two different animal models of tumors. Grapefruit-derived EVs are less toxic than nanoparticles made from synthetic lipids and do not cross the placental barrier when administered intravenously to pregnant mice, making them a promising tool for drug delivery [32].

Grapefruit-derived EVs possess several properties that make them suitable for developing an oral drug delivery system. These properties include biocompatibility, biodegradability, stability in a wide range of pH, and the ability to target specific cells. When combined with the anti-inflammatory drug methotrexate (MTX), the toxicity of the *MTX-grapefruit-derived EVs* was found to be lower compared to free MTX, and the therapeutic effect on mice with DSS-induced colitis was enhanced. This suggests that *grapefruit-derived EVs* could be used as an intestinal immunomodulator and be developed for oral administration of small-molecule drugs to reduce the inflammatory response in human diseases [10].

Ginger-derived EVs can also improve targeting by combining with folic acid, and when loaded with the chemotherapy drug doxorubicin, they showed better efficacy in inhibiting tumors and good biocompatibility at a concentration of 200 μmol/L [6]. Ginger-derived EVs also have better pH-dependent drug release properties compared to commercial liposome doxorubicin [42].

Overall, research has shown that using PDEVs for drug delivery offers many benefits, such as safety and efficacy. Because these vesicles come from natural food sources, they have already been demonstrated to be non-toxic in humans. Moreover, compared to other delivery methods, PDEVs appear to have fewer complications and allow for more specific targeting of cells. Several plant-derived vesicles, including ginger and grapefruit, have been shown to have interesting implications for the delivery of microbial agents and the treatment of intestinal dysbiosis. Additionally, plant-derived vesicles have been found to be incapable of passing the placental barrier, making them promising for drug delivery in pregnant mothers. This evidence suggests that plant vesicles have a wide range of possibilities for future use in health and therapeutics, including the treatment of cancer and other serious diseases. However, more research is needed to explore other edible plant sources for EVs [43].

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