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# Platelet-derived extracellular vesicles for drug delivery

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Platelet-derived extracellular vesicles (PEVs) are a subset of EVs that are released from platelets, which are small nuclear cell fragments that play a critical role in hemostasis and thrombosis. PEVs have been shown to have important roles in a variety of physiological and pathological processes, including inflammation, angiogenesis, and cancer. Recently, researchers, including our group have utilized PEVs as drug delivery platforms as PEVs could target inflammatory sites both passively and actively. This review summarizes the biological function of PEVs, introduces recent applications of PEVs in targeted drug delivery, and provides an outlook for the further development of utilizing PEVs for drug delivery.

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## 1 Introduction

Extracellular vesicles (EVs) are secreted by almost all types of cells, and loading with different cargos, such as proteins and RNAs at nanoscale.[1–3] Compared with cells, EVs are less immunogenic and can penetrate biological barriers due to their nano scale.[4] According to the diameter, EVs can be classified as apoptosis bodies (500–5000 nm), microvesicles (100–500 nm), and exosomes (40–100 nm).[4] Derived from various cells, EVs also comprise membranes, with lipid bilayers and specific proteins from mother cells.[1] For instance, EVs derived from cancer cells contain tumor antigens,[5–7] which have the potential to be utilized as cancer vaccines. EVs derived from red blood cells contain the expression of CD47,[8] endowing EVs with long circulation time.

PEVs were first discovered in 1967 and were considered "platelet dust" at that time.[9] Since then, PEVs have been found with various functions, including the involvement in inflammation and immune system responses.[10] The preparation protocols of PEVs have also been well established.[11] Among various extracellular vesicles, platelet-derived extracellular vesicles (PEVs) are one of the most abundant extracellular vesicles, as more than 50% extracellular vesicles in the blood are derived from platelets and megakaryocytes. PEVs are released from activated platelets, coated with the membrane derived from platelets, with CD41, P-selectin, and other platelet-specific proteins.[12] Because of these specific proteins, PEVs could play an important role in realizing the function of plate-

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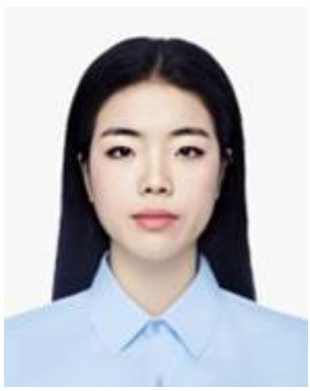

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lets, including hemostasis,[13] immune regulation,[14] and tumor progression.[15] They could also mediate the interaction with different cells, including both immune cells and non-immune cells.

Researchers, including our group, have utilized PEVs as drug delivery platforms as PEVs could target some inflammatory sites both passively and actively. These extracellular vesicles comprising platelet-specific proteins, such as P-selectin, and CD40L, are endowed with the capacity to actively target damaged vessels and activated endothelial cells,[16] which is related to inflammatory diseases and tumors. Compared with platelets, PEVs with their nanoscale, could also passively penetrate through vessels readily and accumulate at the disease site. In addition, PEVs have less immunogenicity, which is possible for allotransplantation. Importantly, when compared with other extracellular vesicles, PEVs could be prepared on a large scale. The exact percentage of platelet-derived EVs varies from 30 to 85%,[17] making it possible for clinical translation. In this review, the biological function of PEVs will be summarized and recent applications of utilizing PEVs in drug delivery will also be reviewed.

## 2 Biology and function of PEVs

PEVs are membrane-coated nanovesicles secreted by activated platelets, in order to communicate with other cells and modulate the function of these cells. Similar to platelets, PEVs could also play a role in hemostasis, inflammatory diseases, and tumor progression. In this part, we will systemically illustrate the biosynthesis, structure, and composition of PEVs, and introduce the role of interaction with cells, and the role of PEVs in various diseases.

### 2.1 Biosynthesis of PEVs

In a natural environment, the initiation of coagulation is usually followed by platelet activation.[18–21] Platelets are reported to be activated mainly through two pathways, G protein-coupled receptors (GPCR) and immunoreceptor tyrosine-based activation motif (ITAM) signaling.[21] Some classical agonists, including thrombin, and ADP activate platelets through the first pathway.[18,20] The activated platelets then release tremendous PEVs to communicate with other cells, including other platelets, endothelial cells, monocytes, and neutrophils, in order to regulate other immune cells and modulate the function of these downstream cells. The formation of PEVs could also originate from cell death, either programmed death platelets, or neurosis platelets.[22]

The actual process of PEV-release has not been fully understood yet,[23] but it could be simply divided into two different ways, one is released from multivesicular endosomes and α-granules, and the other is directly released from the cytoplasmic membrane.[24] In detail, when the platelets are activated, the membrane of the platelets is indented, containing some membrane proteins, and become early endosomes.[25] Then, endosomes selectively load other cargo, including proteins and nucleic acids, from cells. Due to the indention of vesicles, endosomes will gradually turn into multivesicular endosomes (MVE). At the time MVE fuse into the platelet membrane, the small vesicles inside it have been successfully released from platelets.[23] On the other hand, the concentration of cytosolic $Ca^{2+}$ increases after the activation of platelets, causing the reorganization of cytoskeletons, the loss of membrane asymmetry, the blebbing membrane, and finally the production of PEVs.[26,27] During programmed death, PEVs could directly originate from membrane blebbing,[22] which is common in programmed death cells.[28,29]

To produce PEVs on a large scale, scientists stimulate platelets with thrombin or calcium chloride in the imitation of the natural biosynthesis of PEVs. For example, Ma *et al.* activated platelets with 0.5 U thrombin in the incubation at 37 °C.[30] Aatonen and colleagues utilized 2 mmol calcium chloride to activate platelets with high output.[11] Apart from chemical stimulation, PEVs could also be produced by physical methods, such as freeze-thawing. Graça *et al.* produced PEVs by three freeze-thawing cycles of platelet concentrates.[31] This method seems to produce PEVs with higher efficacy, but the proteins and other cargos in produced PEVs need further investigation.

### 2.2 The structure and composition of PEVs

Similar to most extracellular vesicles, PEVs also seem to be heterogenous, as the cargos packaged inside PEVs are not usually similar.[24,32] However, it is still unclear how to regulate the cargo inside PEVs. Generally, PEVs are coated by phospholipid bilayers, derived from platelet membranes, and carry RNAs, proteins, and lipids from platelets (Fig. 1). The existence of CD9, CD63, and CD81, the distinctive proteins of extracellular vesicles, has been detected on the surface of PEVs.[16] CD41 and P-selectin have been identified on most PEV surfaces.[33] Phosphatidylserine could be found on some PEV surfaces.[34] Platelets also package mRNAs and non-coding RNAs inside PEVs to interact with other cells.[35,36] Previous studies have reported the presence of chemokines, cytokines, growth factors, and mitochondria inside some PEVs.[24,37]

### 2.3 Interaction between PEVs and different cells

After release from platelets, PEVs enter the circulating system, and have the opportunity to interact with various cells (Fig. 1). A previous study reported that the interaction between PEVs and endothelial cells, MSCs, smooth muscle cells, platelets, and immune cells could modulate the functions of these cells. Antich-Rosselló and colleagues have confirmed that PEVs are capable of inducing MSCs to osteogenic differentiation, showing the potential of utilizing PEVs in bone regeneration.[38] PEVs could also induce smooth muscle cells towards pro-inflammatory phenotype, mainly through the interaction of CD40 and P selectin.[39] Miyazawa *et al.* have reported that PEVs derived from apheresis platelets could enhance endothelial permeability by increasing the expression of ZO-1 and VE-cadherin after the induction of thrombin.[40] Furthermore, PEVs are also reported to activate platelets and modulate their func-

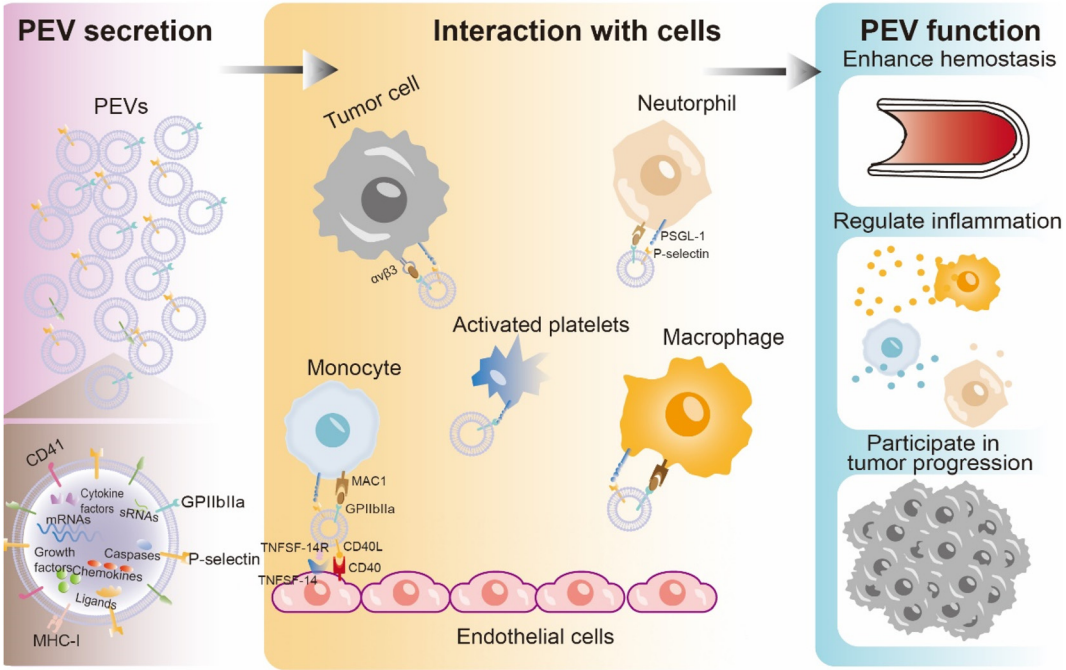


**Fig. 1** Schematic diagram of the structure of PEVs and biological function of PEVs.

tion by generating superoxide due to the expression of Nox-1 on the surface of PEVs.[41]

Apart from the regulation of non-immune cells, PEVs could also modulate various immune cells, and regulate the immune response. P selectin expressed on the surface of PEVs and chemokines loaded in PEVs have been reported to mediate neutrophil recruitment and activation.[42] Additionally, P selectin and these CXC chemokines could further enhance the adhesion of neutrophils to endothelial cells. The interaction between PEVs and monocytes could also enhance the recruitment of monocytes.[42] It has been reported that through the mediation of P selection, GPIbα, the platelet adhesion receptor could be progressively transferred from PEVs to monocytes.[43] Consequently, these GPIbα$^{+}$ monocytes were endowed with the capacity to recruit in damaged vessels and bind to endothelial cells, just like natural platelets.[43] Sadallah *et al.* reported that PEVs could also induce the polarization of macrophages towards the anti-inflammatory stage and promote the maturation of dendric cells derived from monocytes.[44] Moreover, PEVs could also regulate adaptive immune response as regulatory T cells are confirmed to produce less IFN-γ and IL-17 and differentiate towards an inflammatory stage after cocultured with PEVs.[45]

### 2.4 The role of PEVs in hemostasis

Derived from platelets, PEVs could also promote hemostasis and coagulation and were confirmed to stimulate coagulation 50 to 100 times compared with platelets.[46] This may contribute to the high expression of CD41 on the surface of PEVs, which could produce thrombin *via* the tissue factor.[47] It could also be attributed to the higher exposure of phosphatidylserine on PEVs compared to platelets.[48] Lopez *et al.* demonstrated that PEVs isolated from donors could reduce blood loss in rat models, and improve the blood pressure and base deficit as compared with fresh platelets.[49] These results may be attributed to the interaction between PEVs and endothelial cells, as mentioned before. Dyer and colleagues have reported that PEVs released after trauma could promote hemostasis and also promote the formation of thrombosis.[50] Apart from acute wounds, PEVs have also been confirmed to promote chronic wound healing in diabetic rat models, through the activation of Yes-associated protein (YAP).[51]

### 2.5 The role of PEVs in inflammatory diseases

PEVs seem to play both pro-inflammatory and anti-inflammatory roles in inflammatory diseases. On the one hand, PEVs induce the recruitment of innate immune cells, such as monocytes, neutrophils, and promote the release of some cytokines, such as IL-8, TNF-α by monocytes, further modulating the immune microenvironment. French *et al.* reported that PEVs could modulate the bone marrow microenvironment in the LPS-induced sepsis model.[52] PEVs were reported to infiltrate into bone marrow 20 hours after inflammation, and increase the level of megakaryocytes, consequently, restoring hematopoiesis within a short time after inflammation. On the other hand, PEVs could promote lung vasoocclusion under an inflammatory environment. Vats *et al.* found that in the mouse model of sickle cell disease after the challenge of LPS, PEVs carrying IL-1β and Caspase-1 were generated under the induction of platelet-inflammasome activation.[53] This would further induce platelet-neutrophil aggregation in the lung, leading to lung vasoocclusion.

Platelets would be activated under acute infection, including both virus infection and bacterial infection.[14,54] The activation of platelets could be induced by direct interaction of the virus and the interaction of activated neutrophils, followed by the release of PEVs.[55] For instance, once exposed to SARS-Cov-2 *in vitro*, platelets would internalize the virions, and SARS-Cov-2 RNAs could be detected in platelets.[56] This results in the programmed death of platelets together with the release of PEVs.[57] As PEVs are tightly associated with the activation of platelets, which plays an important role in thrombogenesis, thrombin, and inflammation, PEVs could also play the role of a biomarker in patients with infectious diseases, predicting the severity of diseases. The level of PEVs on the whole was reported to be much higher in SARS-Cov-2 positive patients when compared with SARS-Cov-2 negative patients and healthy donors.[58,59] Circulating PEVs could also be a biomarker for HIV-infected patients[60] and patients suffering from dengue virus infection,[61,62] predicting their clinical outcomes.

### 2.6 The role of PEVs in tumor progression

PEVs are generally regarded as cancer-promoter, but they also have the antitumor capacity in tumor progression.[37,63,64] These extracellular vesicles have been reported to play a role in tumor metastasis, regulation of immune cells, and the formation of cancer-associated thrombosis.[37,63,64] PEVs could interact directly with cancer cells, and modulate the phenotype of cancer cells. Yao *et al.* reported that breast cancer cells would differentiate into a metastatic phenotype after the interaction of PEVs, due to the delivery of TPM-3 mRNA in PEVs.[65] Apart from breast cancer cells, PEVs have also been demonstrated to induce the metastasis of prostate cancer cells[66] and lung cancer cells,[15] through upregulation of the expression of MMP-2 in cancer cells. PEVs could also transfer CXCR4 to unexpressed tumor cells, confirmed in various solid tumor cell lines.[67] CXCR4 is a chemokine receptor, stimulating tumor cell growth and migration through various downstream pathways.[68,69] On the other hand, however, Michael and colleagues demonstrated that miRNAs transferred by PEVs could induce the apoptosis of Lewis lung carcinoma cells and suppress tumor growth in vivo.[70]

As mentioned earlier, PEVs could modulate the functions of a variety of immune cells, including monocytes, neutrophils, dendric cells, macrophages, and regulatory T cells, but the direct effect of PEVs on cancer-associated immune cells has not been explored yet. A circulating tissue factor (TF), is usually enriched in the surface of microparticles derived from various cells, which is regarded as a promoter of thrombosis in different tumor models.[71,72] As PEVs also contain abundant TF, they could be a biomarker to predict the risk of thrombosis in cancer patients.[13] However, there is insufficient evidence to directly show the effect of PEVs on cancer-associated thrombosis in vivo.[73]

## 3 The applications of PEVs in drug delivery

As traditional drug delivery systems have limited drug efficacy together with unexpected side effects, drug delivery systems based on nanoparticles have become a part of the next generation of drug delivery technology.[3,74,75] PEVs, also on a nanoscale, could load either hydrophobic drugs inside the phospholipid bilayer or hydrophilic drugs inside the vesicles. Additionally, inheriting the expression of "CD47" from platelets, PEVs were reported to circulate in the body longer than artificial nanoparticles. Similar to most nanoparticles, PEVs could also passively target tumor sites due to enhanced permeability and the retention effect. The most distinctive capacity of PEVs is to actively target damaged vessels because of the expression of P-selectin and other binding proteins. Due to these features, researchers utilized PEVs to deliver various drugs to treat diseases, including cancers, infectious diseases, and autoimmune diseases (Table 1).

### 3.1 PEVs delivery system in infectious diseases

Pathogens, including viruses, fungi, and bacteria, have caused tremendous infectious diseases in the world and led to billions of death every year.[76] For example, SARS-Cov-2, a coronavirus, caused acute pneumonia, leading to nearly 7 million deaths worldwide, as reported by WHO (**https://covid19.who.int/**).[77]

**Table 1** The summary of PEVs for drug delivery

| Diseases | Loading drugs | Mechanisms | Effects | Ref. |
|---|---|---|---|---|
| Acute pneumonia | TPCA-1 | Inhibit IKK | Relief over inflammation in the lung | 30 |
| | Dexamethasone | Reduce the expression of COX-2 | Reduce adverse effects | 84 |
| HIV infection | Lamivudin, Tenofovir diproxil fumarate | Inhibit reverse transcription | Inhibit HIV-1 replication *in vitro* | 85 |
| Atherosclerosis | MCC950 | Inhibit the NLRP-3 inflammasome | Reshape the immune microenvironment | 88 |
| | MSC-EVs | Decrease endothelial dysfunction | Reprogram M1 macrophages to M2 macrophages | 94 |
| Rheumatoid arthritis | Berberine | Regulate ROS-mTOR | Suppress the ankle swelling | 98 |
| Cancer | Paclitaxel | Inhibit cell proliferation | Inhibit the invasion of various tumor cells | 33 |
| | Doxorubicin | Induce cell apoptosis | Show high cytotoxicity on tumor cells | 102–104 |

Pathogen infection accompanies inflammation in the infectious site, and the overwhelmed inflammation may cause a cytokine storm, which could destroy the function of specific tissues, leading to death or irreversible tissue injury.[78] Therefore, how to calm the cytokine storm after acute infection has become an emerging issue. Our group utilized PEVs loading TPCA-1, an inhibitor of IKK, to treat acute pneumonia induced by exposure to LPS (Fig. 2a).[30] The inhibitors of IKK are widely utilized for anti-inflammatory therapies, but the systemic adverse effects hinder their further development.[79,80] This strategy successfully transformed the hydrophobic drugs to a hydrophilic state, with about 15% loading percentage, and delivered drugs to immune cells both *in vitro* and *in vivo*. 20 hours after the treatment of TPCA-1-PEV, the injury, and edema of the lung were relieved almost to the healthy level, evaluated by the level of ROS and the lung wet/dry weight ratio (Fig. 2c). The infiltrating lymphocytes were reduced by nearly 75% after the treatment. The cytokine storm after the exposure to LPS also subsided after the treatment of PEV-TPCA-1, with about 60% reduction of IL-6, TNF-α, and IL-1β (Fig. 2b).

Some glucocorticoids, such as dexamethasone, have been reported to relieve hyperinflammation in the lungs after infec-

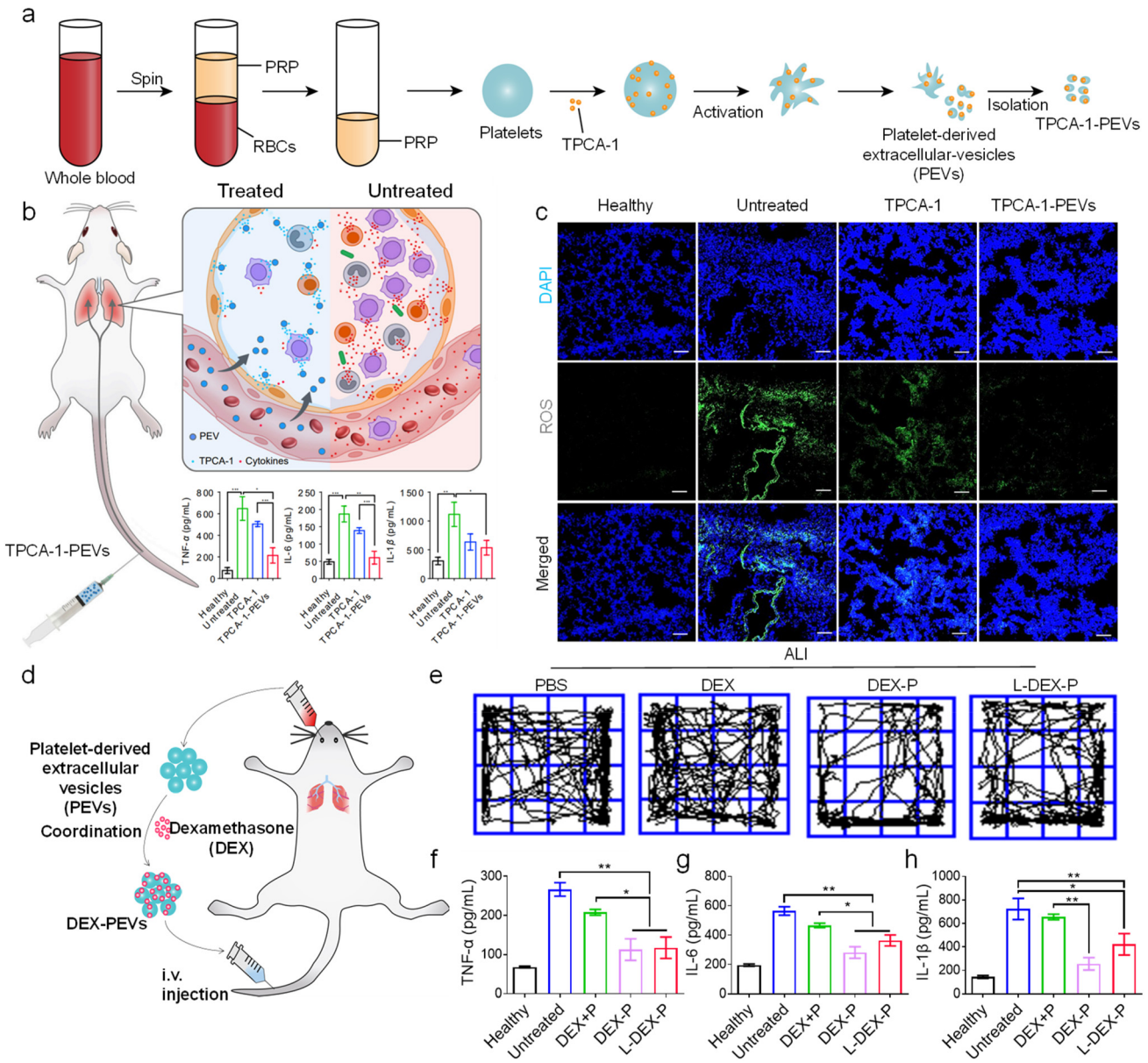


**Fig. 2** The design of anti-pneumonia drug-loaded PEVs. (a) Scheme of the preparation of TPCA-1 loaded PEVs. (b) Schematic of TPCA-1-PEVs for calming down the cytokine storm after acute pneumonia. (c) Anti-pneumonia results of TPCA-1-PEVs evaluated by ROS imaging. Scale bars, 50 μm. Reproduced with permission.[30] Copyright 2020, Elsevier. (d) Scheme of the preparation of DEX-loaded PEVs. (e) The representative paths of mice in the open field test ($n$ = 3). (f–h) Inflammatory factors including TNF-α (F), IL-6 (g), IL-1β (h) of lung tissue after various treatments ($n$ = 3–5). *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.005 (Turkey post-test). Reproduced with permission.[84] Copyright 2021, Royal Society of Chemistry.

tion with SARS-Cov-2.[81,82] But the limited arrival of drugs in the desired location leads to severe side effects and the limited utilization of dexamethasone in the treatment of COVID-19.[83] Ma *et al.* successfully overstrode this obstruction utilizing PEVs to load dexamethasone to treat pneumonia (Fig. 2d).[84] This drug delivery system could significantly increase drug targeting in the desired site, attributed to inflammatory targeting of PEVs. Additionally, this strategy could also reduce the cytotoxicity of dexamethasone by reducing the dosage, including anxiety-like behavior, liver damage, and kidney damage (Fig. 2e). The concentrations of ALT, AST, and CK in the DEX-PEV-treated group were 50% lower than those in the DEX-treated groups (Fig. 2e). PEVs loading a quarter dose of dexamethasone could also relieve the cytokine storm followed by the exposure of LPS, suggesting a high efficacy of PEVs to deliver drugs (Fig. 2f–h). These two strategies have confirmed the potential of drug delivery systems based on PEVs, but further study should be taken to find out the therapeutic efficacy of these drug delivery systems on large animals.

Apart from acute pneumonia caused by pathogens, other infectious diseases, such as acquired immunodeficiency syndrome (AIDS) have also become a worldwide health problem. Soleymani and colleagues designed an anti-HIV-1 drug delivery system based on PEVs, loading both hydrophobic and hydrophilic antiviral drugs.[85] In this study, the encapsulation of these two drugs reached 40% and 60%, respectively. This strategy was confirmed to successfully inhibit HIV-1 replication *in vitro* with limited cytotoxicity compared to free drugs.

### 3.2 PEVs delivery system in autoimmune and autoinflammatory diseases

Apart from inflammation caused by pathogens, people are also suffering from inflammatory diseases caused by disorders of the immune systems. Autoinflammatory diseases are mainly

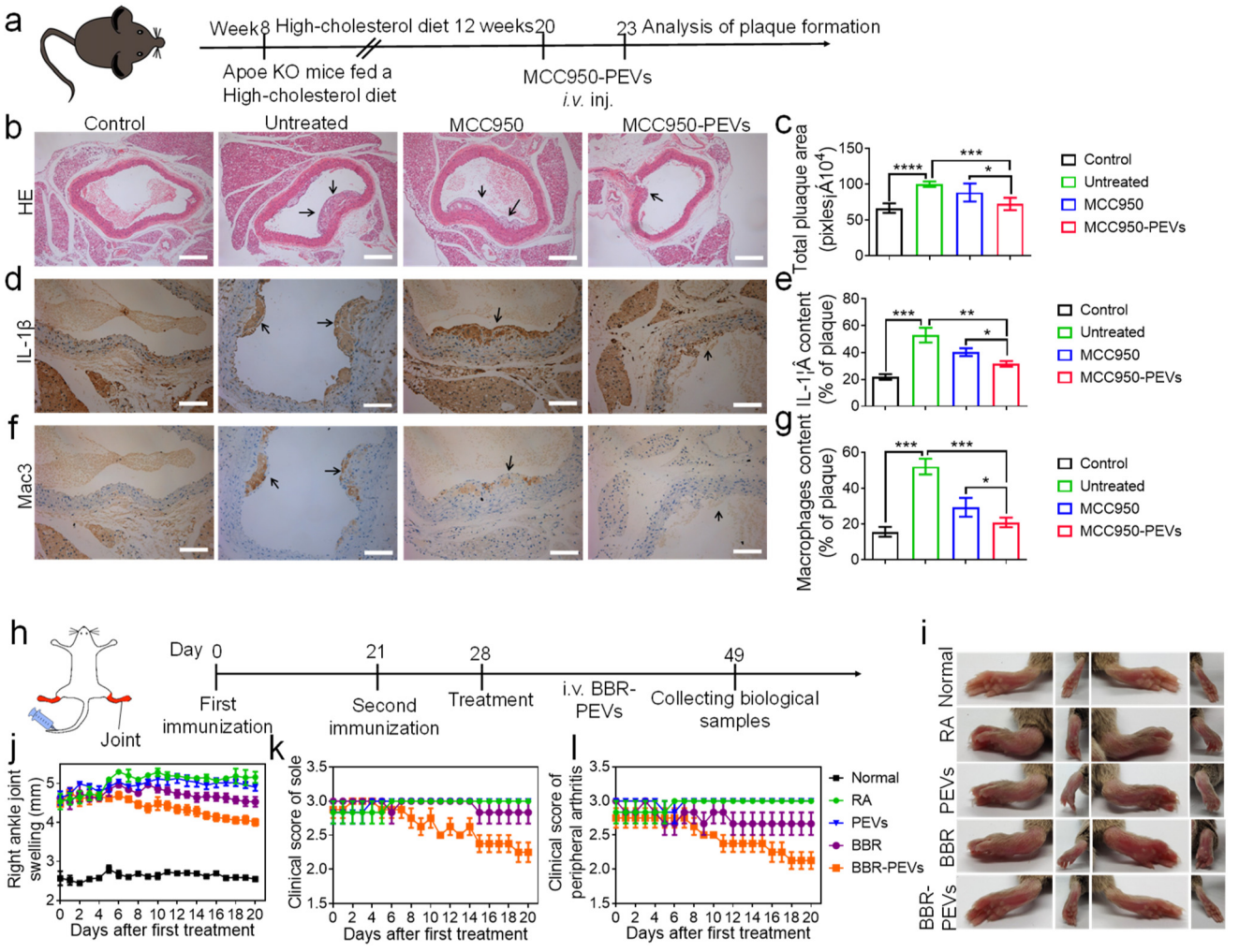


**Fig. 3** Utilizing drug-loaded PEVs in autoimmune and autoinflammatory diseases. (a) Schematic of MCC950-PEVs in atherosclerotic therapy. The staining of the aorta section by H&E (b and c), IL-1β (d and e), Mac-3 (f and g) and quantitative results ($n$ = 3–5). *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.005 (Turkey post-test). Reproduced with permission.[88] Copyright 2021, Elsevier. (h) Schematic of BBR-PEVs in RA model. (i) Hind paws of mice 49 days after the first immunization. (j–l) Clinical score and swelling of mouse paws during the treatment. Reproduced with permission.[98] Copyright 2021, Wiley-VCH.

connected with the dysregulation of the innate immune response, characterized by the enrichment of monocytes, macrophages, and neutrophils.[86] Common autoinflammatory diseases include atherosclerosis, autoinflammatory skin disorders, and early-onset enterocolitis. Atherosclerosis is a main vascular disease, characterized by the accumulation of smooth muscle cells, macrophages, and lymphocytes in the artery.[87] Therefore, modulating the uncontrolled inflammation in the vascular system has become a strategy to treat atherosclerosis. Considering this aspect, our group developed a novel drug delivery system loaded with MCC950 on PEVs to treat atherosclerosis (Fig. 3a).[88] MCC950 is a small-molecule inhibitor of the NLRP3 inflammasome, which has been validated in different animal models.[89–91] Clinical evidence has shown the liver toxicity of MCC950 in RA patients, indicating the need for targeted delivery.[92] This drug delivery system could successfully accumulate in the inflammatory artery and reshape the immune microenvironment in the affected site. After 3 times of infusions, MCC950-PEVs reduced the size of the plaque by about 30%, compared with the untreated group (Fig. 3b and c). The excessive expression of IL-1β in the artery could also be reduced remarkably after MCC-950-PEV treatment (Fig. 3d and e). The macrophage content in the inflammatory artery reduced by nearly 70% after MCC850-PEV treatment (Fig. 3f and g), compared to the untreated group, indicating relief of plaque inflammation. Overall, this study demonstrated PEV as a potential platform for inflammatory targeting drug delivery.

In addition to utilizing pure PEVs as a drug delivery platform, researchers also fabricated PEVs with extracellular vesicles derived from other cells to treat autoinflammatory diseases. Li and colleagues hybridized PEVs with MSC-EVs loaded with miRNA and active factors to treat myocardial ischemia reperfusion.[93] The participation of PEVs endows the system with the targeting ability to damage vasculatures and the pro-angiogenesis potential. This strategy also utilizes atherosclerosis treatment, owing to the plaque targeting of PEVs.[94] However, as the resources of MSCs are various,[95] the quality control of these hybridized EVs should be employed.

Autoimmune diseases including rheumatoid arthritis (RA), and systemic lupus are mainly characterized by uncontrolled adaptive immune responses in specific organs or in the whole system.[96] Glucocorticoids, a common drug utilized to calm the excessive inflammation in autoimmune diseases clinically, also have adverse side effects, such as depression, and immune suppression.[97] Ma and colleagues developed an RA-targeted drug delivery system based on PEVs.[98] Loaded with Berberine (BBR), an alkaloid drug derived from Coptis, PEV-BBR could successfully accumulate in the damaged joints by intravenous injection and reshape the inflammatory microenvironment of damaged joints. The ankle swelling of mice was remarkably suppressed after the treatment of BBR-PEVs, with an 18.4% reduction compared to the untreated group (Fig. 3i–l). This novel drug delivery system could also induce systemic immunosuppression with the reduction of immune cells and the increase of pro-inflammatory cytokines in blood. Therefore, this strategy has great potential to treat systemic inflammatory diseases with easy injection.

### 3.3 The utilization of PEVs in cancers

Apart from inflammatory diseases, PEVs have also been utilized to deliver chemotherapeutic drugs to cancers. PEVs could target tumor sites both passively and actively. PEVs could escape from immune clearance by expressing a "don't eat me" signal, and also circulate for a long time.[10] The passive targeting ability of PEVs could partially be attributed to the EPR effect due to the nanoscale of PEVs.[99] Actively, because of the expression of P-selectin on the surface of PEVs and P-selectin ligands (PSGL) on the surface of tumor cells, PEVs were confirmed to selectively bind towards tumor cells instead of other normal cells.[33] Loading with paclitaxel, PEVs could success-

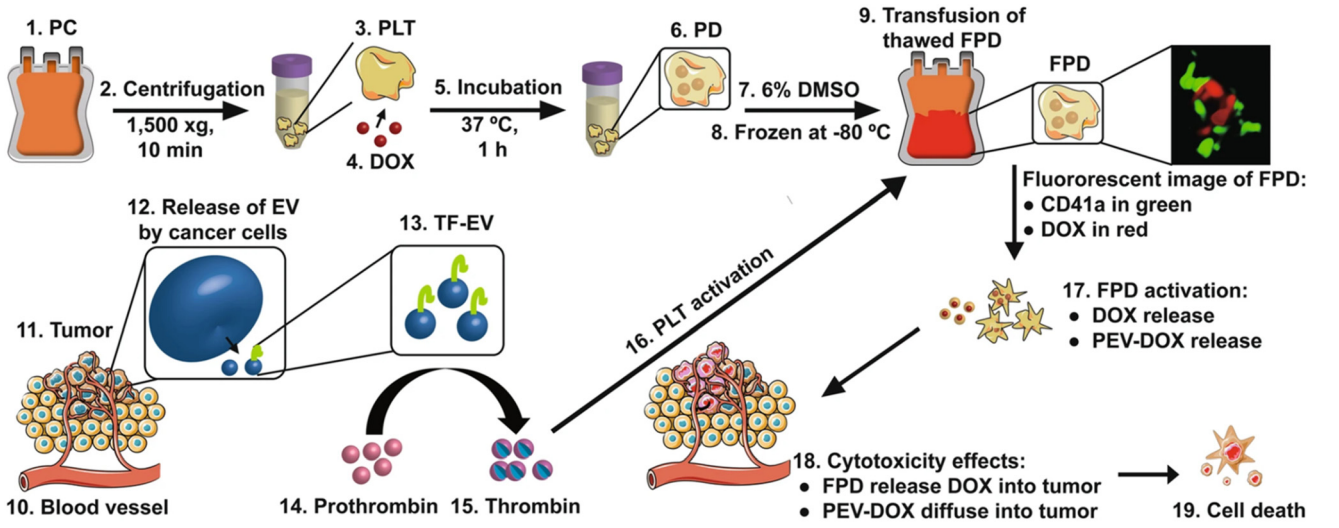


**Fig. 4** The schematic of utilizing PEVs as a drug delivery platform for tumor diseases. Reproduced with permission.[104] Copyright 2020, Springer Nature.

 

fully inhibit the invasion of various tumor cells *in vitro*, including breast cancer cells, and reduce the toxicity of these two chemotherapeutic drugs to normal cells.[33] Apart from paclitaxel, doxorubicin (DOX) is another widely utilized chemotherapeutic drug in clinical.[100] DOXIL, the first nano drug based on doxorubicin encapsulated in liposomes, was first on the market in 1995.[101] However, this nano drug delivery system was reported to accompany adverse effects on the digestive system.[101] Therefore, researchers tried to utilize PEVs loading DOX to enhance the tumor affinity of drugs. Darbandi and colleagues utilized PEV-DOX to induce the apoptosis of Daudi cells.[102] Kailashiya *et al.* confirmed that compared with free DOX, PEV-DOX has a higher affinity to human leukemia cells, higher EC50 to tumor cells, together with lower vascular drug release.[103] Additionally, the cytotoxicity of PEV-DOX on breast, colon, and lung cancer cells was also higher than that of free drugs, due to the high affinity of PEV-DOX on tumor cells (Fig. 4).[104] PEVs utilized in the above three studies are derived from human blood, and prepared in fast ways, showing the high potential of clinical translation of PEVs as a drug delivery platform for tumor treatment. Even though the *in vivo* therapeutic efficacy was not confirmed in previous literature, PEVs have shown great potential towards tumor sites and inhibit tumor growth.

## 4 Perspectives

Derived from platelets, PEVs have exhibited the potential to become a novel drug delivery carrier for inflammatory targeting. As PEVs mostly originate naturally without artificial synthesis, these extracellular vesicles show neglectable toxicity with high biocompatibility. Working as a drug carrier, PEVs could be used for loading both water-soluble and water-insoluble drugs with high efficacy. In the nanoscale, platelet-derived extracellular vesicles could penetrate biological barriers *in vivo* and could circulate in the body for a long time, making it possible to passively target affected sites. Additionally, coated by the membranes inherited from platelets, PEVs are endowed with the capacity of actively inflammatory targeting, with the assistance of specific proteins expressed on the surface. Therefore, PEVs are considered ideal carriers for targeting drug delivery.

Recent studies have utilized extracellular vesicles derived from tremendous cells, including red blood cells,[105,106] white blood cells,[107–111] and stem cells[112] for drug delivery. When compared with extracellular vesicles derived from other cells, PEVs have high output from their parental cells as platelets could release abundant extracellular vesicles after activation, while other cells fail to have such a process. Additionally, the inflammatory targeting ability is also distinctive for PEVs. Not only PEVs but platelet-membrane coated nanoparticles are also showing the inflammatory targeting effect when used for drug delivery.[113–118] However, PEVs are derived through a simpler process without any further artificial synthesis, compared with nanoparticle-coated platelet-membrane.

On the other hand, the further development of utilizing PEVs as a drug delivery system is full of opportunities and challenges. To ensure the quality of PEV preparations, various quality control (QC) measures should be employed. In addition, understanding the actual mechanism of PEV release could provide platforms to selectively utilize homogeneous PEVs as drug carriers toward specific targets. It is also possible to produce genetic modifications of megakaryocytes or make a modification of platelets, importing the interested genes or proteins directly. How to produce PEVs on a large scale with great stability and safety is also a challenge for the further development of PEV utilization.

## Conflicts of interest

There are no conflicts of interest to be declared.

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