Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Calming Cytokine Storm in Pneumonia by Targeted Delivery of TPCA-1 Using Platelet-Derived Extracellular Vesicles

Platelet-derived extracellular vesicles were engineered for targeted delivery of anti-inflammation therapeutics to treat pneumonia. This delivery strategy improved therapeutic efficacy, inhibited the pulmonary inflammatory cell infiltration, and calmed local cytokine storm syndromes compared with the free drug-treated group.

HIGHLIGHTS
Platelet-derived extracellular vesicles are used for pneumonia-targeted delivery

Targeting delivery can improve the therapeutic efficacy while reducing side effects

Such technology can be useful for inflammatory disease diagnosis and drug delivery

Ma et al., Matter 3, 287–301
July 1, 2020 © 2020 Elsevier Inc.
https://doi.org/10.1016/j.matt.2020.05.017
Calming Cytokine Storm in Pneumonia by Targeted Delivery of TPCA-1 Using Platelet-Derived Extracellular Vesicles

Qingle Ma,1,4 Qin Fan,1,4 Jialu Xu,1 Jinyu Bai,2 Xiao Han,3 Ziliang Dong,1 Xiaozhong Zhou,2 Zhuang Liu,1 Zhen Gu,3,* and Chao Wang1,5,*

SUMMARY

Pneumonia can cause high morbidity and mortality because of uncontrolled inflammation in the lung tissue. Calming the cytokine storm may be one key to saving the life of patients with severe pneumonia. Here, inspired by the intrinsic affinity of platelets to the site of inflammation, we have engineered platelet-derived extracellular vesicles (PEVs) for pneumonia-targeted drug delivery. It is demonstrated that PEVs that are easily generated from the activated platelets can selectively target pneumonia in the mouse model with acute lung injury (ALI). By loading with [5-(p-fluorophenyl)-2-ureido]thiophene-3-carboxamide (TPCA-1), which can inhibit the production of inflammatory factors, the PEVs significantly improve therapeutic benefits by inhibiting the infiltration of pulmonary inflammatory cells and calming local cytokine storm compared with the free drug-treated group. Furthermore, we find that PEVs could serve as a broad platform that can selectively target various inflammatory sites, including chronic atherosclerotic plaque, rheumatoid arthritis, and wounds associated with skin.

INTRODUCTION

Inflammation as a defense mechanism in the body is an immune response at the basis of many physiological and pathological processes.1–2 However, certain infections can also cause an overwhelming local/systemic inflammatory response, leading to life-threatening diseases such as pneumonia.3 For example, the 2019 novel coronavirus (SARS-Cov-2; COVID-19) has infected more than 3 million people and has killed more than 200,000 worldwide so far.4 The mortality rate in severe patients is greater than 60%.5 Increasing evidence suggests that patients with severe pneumonia have cytokine storm syndrome, whereby the body’s immune response leads to uncontrolled inflammation of lung tissue.6

As far as we know, inhibiting the cytokine storm may be one key to saving the life of patients with severe pneumonia infected with a highly pathogenic virus such as SARS-Cov-2.7–10 Certain anti-inflammatory therapeutics have proved beneficial in clinical treatment.11–13 For example, tocilizumab (an interleukin-6 [IL-6] receptor blocker) and corticosteroids have been approved to treat patients with COVID-19 pneumonia in China. Although promising, current systemic treatment options for inflammation therapy in the clinic often lead to high/frequent dosing and side effects. For instance, osteonecrosis has often occurred in SARS (severe acute respiratory syndrome) patients because of corticosteroid use.14 Targeting the pneumonia to improve the efficacy while reducing the dosage and side effects remains elusive.
in clinical treatment. Further efforts are demanded to develop targeting delivery systems for modulating and reducing the local inflammatory responses in pneumonia.15–17

Cellular delivery systems have raised increasing attention due to their excellent biocompatible and unique delivery behaviors.18–22 Platelets are one kind of inherent cells in the body, which if serving as drug-delivery systems are equipped with several advantages compared with other synthetic delivery systems.19 For example, as one role of immune cells, platelets have intrinsic affinity with the site of inflammation.23–25 They can bind to the activated/inflamed vascular walls through a range of receptor patterns, including CD40L, glycoproteins Ibα, αIIb, and VI, and P-selectin.23–25 In light of this, here we leveraged platelet-derived extracellular vesicles (PEVs) to facilitate the delivery of anti-inflammatory agents to pneumonia upon intravenous administration (Figure 1A). A large number of PEVs can be readily obtained by activating platelets in vitro.26,27 Interestingly, we found that the PEVs showed the excellent capacity of accumulating at the site of pneumonia. PEVs released limited inflammatory factors in the active environment compared with platelets, enabling them as an anti-inflammatory drug carrier. By loading with anti-inflammation agents [5-(p-fluorophenyl)-2-ureido]thiophene-3-carboxamide (TPCA-1), the TPCA-1-PEVs significantly reduced the inflammation and cytokine storm syndromes and relieved symptoms in mice with pneumonia induced by acute lung injury (ALI). The therapeutic benefit of drug-loaded PEVs was significantly enhanced compared with that of drug alone in a mouse disease model. Furthermore, such a PEV-based platform could be made by mixing the activated platelets from the patient and anti-inflammation agents ex vivo, followed by isolation and reinfusion into the same patient for personalized medication.

RESULTS AND DISCUSSION

Cytokine Storm in Mouse with Acute Lung Injury

Lung is a major source of cytokine storms in patients with pneumonia infected by highly pathogenic viruses such as SARS-Cov-2, which can result in high morbidity and mortality. In this study, we first evaluated the cytokine storm in the lung following the ALI of mice. ALI can be caused by pathogens, such as influenza A virus H5N1 and SARS-CoV-2,28 which is characterized by excessive inflammatory response in the lungs that induce the dyspnea and terminal failure of the respiratory system as well as acute respiratory distress syndrome (ARDS).27 It is validated that overwhelming inflammatory reactions lead to varying degrees of lung injury.30,31 As a result, we confirmed that high CD45 immune cells infiltration (Figures S1A and S1B) and a high level of pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α), IL-6, and IL-1β production (Figures S1C and S1E) in the lung tissue, were induced in ALI mice.

Preparation and Characterization of PEVs

We prepared the targeted delivery platform using the PEVs (Figure 1A). A large number of platelet-derived vesicles can be generated and released after platelet activation.32,33 Here, we used thrombin to activate the platelets in vitro. Pre- and post-activated platelets and PEVs were analyzed by transmission electron microscopy (TEM) (Figures 1B and 1C) and dynamic light scattering (DLS) (Figures 1D–1F and S2A). The activated platelets exhibited a size of 100–200 nm as shown in DLS data compared with platelets without activation, which indicated PEV generation after activation (Figure 2E). PEVs were then isolated from the platelet-activated supernatant by ultracentrifugation according to a standard protocol.32 The purified PEVs showed a spherical shape with sizes of about 100–150 nm (Figure 1C) compared with the
non-activated platelets (Figure 1B). To ensure the obtained vehicle was derived from platelets, we examined protein profiles of platelets and PEVs by SDS-PAGE and western blot. As expected, PEVs partially contained proteins from original platelets (Figure 1G). The existence of CD41 on PEVs further confirmed that PEVs were derived from platelets, while some cytosolic proteins such as actin was lost in PEVs compared with platelets (Figure 1H). Besides, PEVs were stored in various buffers at 4°C for at least 4 days in vitro (Figure S2B). In the absence of platelet aggregation inhibitor prostaglandin E1 (PGE1), the size of PEVs did not change significantly, indicating the high stability of PEVs ex vivo.

As pro-inflammatory cells, platelets may also accelerate inflammation and progression by the release of inflammatory factors when binding to the disease site.34 To determine whether PEVs also release pro-inflammatory cytokines upon activation, an ELISA was used to detect the IL-1β and IL-6 in the supernatant via thrombin activation in PBS. Unlike platelets, PEVs did not release cytokines significantly after thrombin treatment (Figures 1I and 1J), suggesting that PEVs could not aggravate the inflammatory response after transporting to the inflamed site compared with the naive platelets.27 These results suggested the promise of PEVs as a drug carrier for inflammation disease targeting. We further tested the hemostatic capacity of PEVs in vivo. The time of tail-bleeding after transection was monitored after pre-injection of PBS, platelets, or PEVs at a dose of about 12.6 mg/kg determined by

Figure 1. Characterization of the Platelet-Derived Extracellular Vesicles

(A) Scheme of the preparation of drug-PEVs. PRP, platelet-rich plasma; RBCs, red blood cells. TPCA-1-PEVs secreted by platelets were collected and purified by ultracentrifugation.

(B and C) Morphology of the platelet (B) and PEVs (C) observed by TEM. Scale bars, 1 μm.

(D–F) Particle size distribution measured by DLS in non-activated platelets (D), activated platelets (E), and PEVs (F).

(G) SDS-PAGE of platelet lysate and PEVs with Coomassie brilliant blue staining.

(H) Western blot analysis of CD41 and actin from the platelet lysate and PEVs.

(I and J) Amount of IL-1β (I) and IL-6 (J) cytokines from platelets and PEVs in the absence and presence of thrombin.

(K) The hemostatic effect of constructs in the mouse tail transection bleeding model.

Data are means ± SEM (n = 3–5). Statistical significance was calculated by one-way ANOVA using Tukey’s post test. **p < 0.01; n.s., not significant.
bicinchoninic acid analysis. Our results showed that no significant hemostasis occurred after administration, indicating that the PEVs could not increase the risk of hemostasis after intravenous injection at the current dose (Figure 1K).

**In Vitro Targeting of Blank PEVs to Inflammatory Cells**

To mimic the inflammatory microenvironment, we converted RAW264.7 cells to activate macrophages by lipopolysaccharide (LPS) treatment. The activated macrophages were then incubated with platelets or PEVs labeled with 1,1-dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine (DiD) (Figures 2A and 2B). Platelets were used as a positive control. It was observed by fluorescence imaging that, similar to the naive platelets, PEVs showed a higher affinity toward activated cells compared with the non-activated macrophages (Figures 2A and 2B). The targeting effect was further observed in activated endothelia, which also play a dominant role in initiating the process of inflammation (Figures 2C and 2D). Both platelets and PEVs could...
target human umbilical vein endothelial cells (HUVECs) induced by LPS. This binding ability was significantly weakened on resting activated cells or unactivated endothelial cells. Collectively, these results indicated that PEVs also had affinity with the major components of inflammation-associated cells in vitro. Nevertheless, receptors/proteins between the PEVs and inflamed cells as well as the mechanism of vesicle internalization by inflamed cells need to be further investigated in detail.

**Accumulation of PEVs Targeted toward Acute Lung Injury**

We then investigated whether PEVs could specifically target inflammatory lungs in vivo (Figure 2E). Free DiD or DiD-labeled PEVs (at the same dose of DiD determined by absorption, PEVs: 12.6 mg/kg) were injected in the ALI mice intravenously. After injection for 2 h (Figures 2F–2I), the lungs were collected and imaged by an ex vivo imaging system. Interestingly, we observed that DiD-PEVs showed the strongest fluorescence intensity in the affected lungs compared with those in healthy mice or free dye-treated ALI mice (Figures 2F and 2G). Of note, in the DiD-PEVs-treated healthy mice or DiD-treated ALI mice at the 2-h time point, most of the signals were located in the liver. In contrast, the major organ of DiD distribution was the lung in the DiD-PEVs-treated ALI mice (Figures 2H and 2I), suggesting excellent accumulation capacity of PEVs at the acute lung inflammation site. The confocal images of lung tissue also showed that enrichment of PEVs compared with free DiD treatment or in normal lungs (Figure 2J), further confirming the targeting capacity of PEVs to the injured lung tissue.

These results can be explained by the intrinsic affinity of PEVs to the site of inflammation. PEVs were able to bind to the activated/inflamed vascular walls through a range of receptor patterns, including CD40L, glycoproteins Ibα, αIIb, and VI, and P-selectin.23–25 In addition, nanosized PEVs can also passively target the inflamed lung tissue: temporary dilated and leaky blood vessels caused by histamine in response to injury allowed injected nanosized PEVs to pass through the vasculature and reach the injured tissue.38

**Acute Pneumonia Treatment by TPCA-1-PEVs Infusion**

We then hypothesized that PEVs were a platform delivery carrier for targeting drug delivery toward inflammation. TPCA-1, a potent and selective inhibitor of IκB kinases (IKK) in nuclear factor (NF)-κB pathway, can inhibit the production of TNF-α, IL-6, and IL-8 from human monocytes39,40 (Figure 3A). It has been demonstrated that TPCA-1 can reduce the severity of pneumonia in mouse.41 Here, we loaded the TPCA-1 into the platelets, likely by means of passive diffusion and/or hydrophobic interaction, followed by platelet activation to obtain the TPCA-1-PEVs. The characteristic absorption peak of TPCA-1 in the absorption spectrum indicated the successful loading of TPCA-1 into the PEVs (Figure 3B). The size and the zeta potential of the TPCA-1 loaded PEVs did not change significantly compared with empty PEVs (Figure S3). The drug-loading percentage was about 10.6% (loaded/added TPCA-1) (Figure 3C) and we obtained TPCA-1-PEVs containing 4.6 wt % of TPCA-1 at the TPCA-1 concentration of 600 μg/mL. The PEVs exhibited a controlled and sustained release profile, with approximately 85.3% of total TPCA-1 leaking out from the PEVs into the medium within 48 h (Figure 3D).

In vitro anti-inflammation therapeutic effect of TPCA-1-PEVs was also confirmed. All treatments were able to reduce the production of IL-6 and TNF-α (Figures 3E and 3F). The level of IL-1β was not affected remarkably (Figure S4), probably due to the negative regulation of NF-κB in IL-1β secretion.42 In addition, we studied the immune response of macrophages after the treatment by TPCA-1-PEVs. As expected,
LPS-stimulated macrophages displayed a typical M1 phenotype. Addition of TPCA-1 or TPCA-1-PEVs into the LPS-stimulated macrophages resulted in a lower level of CD80+, indicating that TPCA-1-PEVs inhibited the inflammatory response and M1 polarization induced by LPS (Figure 3G). Moreover, treatment with TPCA-1-PEVs significantly reduced LPS-induced reactive oxygen species (ROS) production of macrophages (Figure 3H).

To demonstrate the therapeutic efficacy by targeting delivery, we challenged mice with LPS (8 mg/kg) to induce ALI.40 Four hours later, PBS, free TPCA-1 (1 mg/kg), and TPCA-1-PEVs (equal to 1 mg/kg of TPCA-1) were intravenously administered (Figure 4A). All mice were euthanized 20 h after injection for analysis. As the production of ROS during pneumonia may contribute to lung injury, 2,7-dichloro-di-hydrofluorescein diacetate (DCFH-DA) staining of lung tissue for detecting ROS production was performed. As shown in Figure 4B, we found that TPCA-1-PEVs treatment could significantly inhibit the generation of ROS during acute pneumonia, while free TPCA-1 treatment showed a limited ROS-scavenging capability. In addition, the level of malondialdehyde (MDA) and myeloperoxidase (MPO), which are important defenses in neutralizing ROS in the lung, were found to increase remarkably, validating the removal of ROS (Figures 4D and 4E).43

In addition, we measured the lung wet/dry weight ratios to observe lung edema. Pathological examinations have revealed that the lungs of COVID-19 patients exhibited edema, which may be due to the promoted mucus production and immune
cell infiltration. Administration with TPCA-1-PEVs significantly reduced the lung edema compared with free drug treatment (Figures 4F and SS). Moreover, histological examination validated the existence of excessive pulmonary edema, alveolar inflammatory cell exudation/infiltration, and alveolar injury in the untreated ALI group (Figure 4G). In the treatment groups, the inflammatory cell infiltration of mice receiving TPCA-1-PEVs was reduced significantly compared with the untreated ALI group and free drug treatment.

As already mentioned, preventing or inhibiting the cytokine storm may be one of the keys to saving the life of patients with severe pneumonia. Therefore, we investigated
whether the lung cytokine storm could be calmed by targeted delivery of TPCA-1 using PEVs. The levels of TNF-α, IL-6, and IL-1β in lung tissue homogenate were measured by ELISA following the treatments (Figure 4A). Although the TPCA-1 has been demonstrated to decrease the cytokine storm in previous studies, the therapeutic efficacy was limited in our experimental mouse ALI model at the dose of 1 mg/kg. The free TPCA-1 treatment moderately reduced the cytokine level. PEVs alone did not result in reduced immune cell infiltration and cytokine concentration (Figure S6). Encouragingly, these inflammatory factors were significantly declined after treatment with TPCA-1-PEVs, indicating that the cytokine storm was efficiently inhibited by the targeted delivery of TPCA-1 using the PEVs (Figures 5A–5C).

Infiltration of immune cells is a key sign of pneumonia and is associated with cytokine storm. In patients infected with SARS-CoV-2, it has been reported that the severity of pulmonary immune injury correlated with major infiltration of neutrophils, macrophages, and T cells in the lungs.44 We also tested the persistence of immune cells in the lung after treatments, whereby the total percentage of CD45+ cells at the site of lung tissue was reduced remarkably in the mice receiving the TPCA-1-PEVs compared with the TPCA-1 alone (Figures 5D–5F). We next measured macrophages in the lung. CD11blo F4/80hi resident macrophages play crucial roles in ALI. In the acute phase of ALI/ARDS, resident macrophages shift into the classically activated phenotype (M1) and release various potent pro-inflammatory mediators.45–47 We observed a decreased level of the CD11bloF4/80hi macrophage cell subset in mice treated with TPCA-1-PEVs compared with mice treated with free drug (Figures 5G and 5H). Furthermore, a significantly higher percentage of CD14+CD45+ inflammatory infiltrating monocytes/macrophages (Figures 5I and 5J)44,48,49 was found in the untreated mice, while mice injected with TPCA-1-PEVs exhibited a remarkable decrease of CD14+CD45+ inflammatory immune cells. We further analyzed the proportion of CD3+CD45+ T cells in the lung (Figures 5K and 5L), which was significantly lower in mice treated with TPCA-1-PEVs than in those treated with TPCA-1 alone. In addition, H&E staining of other major tissues confirmed that PEVs have limited toxic effects on treated mice (Figure S7).

**Accumulation of PEVs Targeted toward Other Inflammatory Diseases**

Inflammation is associated with various human diseases that affect people’s health and quality of life. Encouraged by our results associated with ALI, we hypothesized that PEVs could be also be applied to target other inflammations. To test our hypothesis, we tested the potency of PEVs targeted to inflammatory sites as a universal strategy in several other inflammation disease models, including atherosclerotic plaque, rheumatoid arthritis, and skin wound. Interestingly, we found that PEVs could selectively target both chronic and acute inflammatory sites in various disease models, including chronic atherosclerotic plaque (Figure 6A), rheumatoid arthritis (Figure 6B), and acute wound injury (Figure 6C) compared with free dyes. Obvious accumulation of DiD-PEVs was noticed at inflamed tissue. Our results indicated that the PEV approach may potentially provide a simple and useful platform technique for the detection of and drug delivery to inflammatory disease.

**Conclusions**

In summary, we have developed a pneumonia-targeting treatment strategy platform based on PEVs. Inspired by the inherent ability of PEVs to target the inflamed site, we found that PEVs could accumulate at the site of inflammation associated with pneumonia and facilitate the delivery of anti-inflammatory agents by intravenous administration. Our results highlighted that targeted drug delivery to treat pneumonia significantly reduced the cytokine storm syndromes when compared with free
Figure 5. Lung Cytokine Storm and Infiltration of Immune Cells in the Lung Tissue Could Be Calmed by Targeted Delivery of TPCA-1 Using Platelet-Derived Extracellular Vesicles

(A–C) Inflammatory factors including TNF-α (A), IL-6 (B), and IL-1β (C) of lung tissue homogenate after various treatments as indicated.

(D) Lung sections were stained with CD45 after various treatments as indicated. Scale bar, 100 μm.

(E and F) Representative plots of CD45+ cells as a percentage of the total cell population (E) and corresponding quantification results (F).
drug therapy. Our formulation may inspire new treatments for COVID-19 patients. Furthermore, we found that PEVs could selectively target various inflammatory sites, indicating that it could serve as a broad platform for inflammation targeting. Due to its excellent biocompatibility and ease of preparation, such technology showed potential for further clinical translation.

EXPERIMENTAL PROCEDURES

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Chao Wang (cwang@suda.edu.cn).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

This study did not generate custom code, software, or algorithms.

Preparation and Characterization of PEVs

Mice platelets were separated according to the protocol reported previously. In brief, whole blood was collected from the sinus of BALB/c mice and resuspended in PBS containing EDTA (5 mM, Sigma-Aldrich) and PGE1 (1 μM, MCE), then centrifuged at 100 x g for 15 min to remove red blood cells. The supernatant was collected and centrifuged at 800 x g for 20 min. Centrifuged sediment was stored at room temperature for further use after resuspension. For the preparation of PEVs, the platelet concentrate was activated by thrombin (2 U/mL, Solarbio) for 30 min and centrifuged at 800 x g for 10 min. The collected supernatant was further subjected to ultracentrifugation to concentrate the PEVs into granules at a rate of 100,000 rpm for 2 h. The morphology of PEVs was detected by TEM. Protein expression of platelet and PEVs was determined by SDS-PAGE and western blot. The particle size and changes over time were measured by DLS. To prepare TPCA-1-platelet, we added TPCA-1 (APExBIO) dissolved at 600 μg/mL in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) at specified inputs directly to platelets suspended in PBS containing EDTA (5 mM, Sigma-Aldrich) and PGE1 (1 μM, MCE) for 12 h. The loading and sustained release of TPCA-1 (APExBIO) on PEVs was detected by high-performance liquid chromatography (HPLC). To test the loading capacity, we measured the loading content by HPLC. The TPCA-1-PEV suspension was dried by lyophilization and weighed. The drug content of TPCA-1 in PEVs was calculated with the following equation: loaded drug/dried TPCA-1-PEVs (w/w). We obtained TPCA-1-PEVs containing 4.6 wt % of TPCA-1 at TPCA-1 concentration of 600 μg/mL. The DiD or TPCA-1 was dissolved in a small amount of DMSO and diluted with PBS for further use.

Animals

BALB/c mice (female, 6–8 weeks old, 18–20 g each) were purchased from the experimental animal center of Soochow University. Mice were allowed to adapt to the laboratory for at least 7 days in climatic conditions. Experimental group sizes were
approved by the regulatory authorities for animal welfare after being defined to balance statistical power, feasibility, and ethical aspects. The experiments were conducted under the guidance of the Institutional Review Board of Soochow University, which complied with relevant ethical codes.

**Cell Lines**

RAW264.7 cells (mouse leukemia cells of monocyte macrophage) and HUVECs were obtained from American Type Culture Collection. The cells were cultured in DMEM solution containing 10% fetal bovine serum (Invitrogen), 100 U/mL penicillin/streptomycin (Invitrogen). Cells were tested every 3 months to exclude the presence of mycoplasma.
**Animal Model Induction and Treatment**
The ALI mouse model was established according to previous reports. BALB/c mice were anesthetized, then fixed in the supine position and injected intratracheally with LPS (8 mg/kg) into the posterior pharyngeal wall. After 4 h, mice were treated once with TPCA-1 or TPCA-1-PEVs. Animals were euthanized 24 h after the last injection.

**MTT Cytotoxicity Assay**
RAW264.7 cells (1 × 10^4 cells/well) were plated on a 96-well plate. After 12 h, this was replaced with a medium containing different concentrations of the drug. After 24 h of incubation, MTT reagent was added and incubated for another 4 h. Thereafter, the supernatant was removed and the residue dissolved by adding DMSO. The absorbance of the solution was recorded with a multi-wall plate reader at 570 nm.

**In Vitro Binding**
To obtain activated macrophages, we cultured RAW264.7 with 100 ng/mL LPS for 24 h. For adhesion studies, activated cells were co-incubated with DiD-loaded platelets or PEVs for 24 h and washed three times with PBS to remove the unbound particles. The cells were stained with DAPI after fixing with 4% paraformaldehyde. The results were obtained by fluorescence microscopy. For activated endothelial cell binding, HUVECs were pre-stimulated with 100 ng/mL LPS (Biosharp) and incubated with DiD-labeled platelet or PEVs, respectively, for 24 h. The unbound particles were removed by washing with PBS three times. The binding behaviors were determined after staining with DAPI.

**Intracellular ROS Detection**
ROS production was detected by a ROS assay kit. To create an inflammatory environment, we pre-treated RAW264.7 cells with 100 ng/mL LPS (Biosharp) for 1 h. TPCA-1 or TPCA-1-PEVs were added in groups and treated for another 24 h. Cells were then cultured with 20 μM DCFH-DA (Sigma) for 30 min at 37°C. Subsequently, the cells were washed three times with serum-free medium. The fluorescence signal intensity of DCFH-DA oxidatively transformed fluorescent dichlorofluorescein was measured by flow cytometry, and the change of intracellular ROS level was measured using FlowJo software.

**Cytokine Assay**
For in vitro stimulation, RAW264.7 cells were pre-treated with 100 ng/mL LPS (Biosharp) for 1 h, then TPCA-1 and TPCA-1-PEVs were added in groups and treated for another 24 h. Cytokine production was analyzed by ELISA according to the cell supernatant provided by the manufacturer.

**Western Blotting**
The protein expression of the inflammatory factor in the cell was detected by western blotting according to the existing method. In brief, cells were lysed with RIPA lysis buffer, added to the loading buffer in proportion to boiling point, and proteins were separated by 10% SDS-PAGE. The wet transfer procedure was followed to transfer the protein to the PVDF membrane. After the milk powder was blocked, incubation with anti-CD41 (1:1,000) and β-actin (1:5,000) antibodies and detection with the horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G secondary antibody (1:5,000) was carried out. FluorChem was used to display hybridization bands, and the software ImageJ was used for quantitative analysis of western blots.
In Vivo Targeting to Inflammatory Site
The model was generated as mentioned above. The mice were injected intravenously with DiD-loaded PEVs. Age-matched normal mice were used as controls. At the pre-determined time intervals, mouse and organ were imaged using IVIS Spectrum Imaging System (PerkinElmer). For histological analysis, the organ was collected and embedded in optimal cutting temperature compound, and frozen sections were sectioned. Each section was 10 μm thick and fixed in 4% paraformaldehyde for 30 min. After washing three times with PBS, 200 μL of serum was added to each section. Subsequently, CD68 primary antibody (Abcam) incubation was carried out, followed by fluorescein isothiocyanate-conjugated secondary antibody incubation. Each part was stained with 200 μL of DAPI after washing three times with PBS containing 0.05% Tween 20. The slides were sealed and analyzed by confocal microscopy.

Histological Analysis and Immunohistochemical Staining
At the end of the experiments, the organ was fixed with 4% paraformaldehyde overnight and embedded in paraffin. Paraffin sections were cut into the slice with a thickness of 4 μm. H&E staining was used to assess inflammatory infiltration. The amount of CD45 was analyzed by immunohistochemistry.

Flow Cytometry
Mice were euthanized and the dissected tissues crushed by tissue grinding. The removed tissue was homogenized by nylon gauze to obtain a single-cell suspension, then treated with red blood cell lysis buffer and stained with fluorescent antibody (BioLegend) as indicated. The stained cells were analyzed on a BD Accuri C6 flow cytometer using the FlowJo software package.

Lung Wet/Dry Ratios
The tissue samples were weighed (wet weight) immediately after removal and dried in an oven at 45°C until a stable dry weight was reached after 48 h. The ratio of wet weight to dry weight was then calculated to quantify the degree of pulmonary edema.

Analysis of Lung Tissue Homogenate
The lung tissue was weighed and 10% of tissue homogenized with PBS as the homogenization medium. The MPO and MDA tests followed the steps of the kit manufacturer. Inflammatory factors were detected by ELISA according to the manufacturer’s protocol.

Statistical Analysis
All results are expressed as mean ± SD or mean ± SEM. Repeated groups were included in all experiments unless otherwise stated. When the data of the two groups were compared, the results were significant (p < 0.05). For multiple comparisons, Tukey’s post hoc test for statistical differences was used. All statistical analyses were performed using GraphPrism (v5.0). Significance in the figures is indicated by asterisks: *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001. All intensities of fluorescence expression in the experiments were further calculated by ImageJ software.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.matt.2020.05.017.
ACKNOWLEDGMENTS
This work was supported by grants from start-up packages of Soochow University and the Program for Jiangsu Specially-Appointed Professors to C.W. This work is partly supported by the Collaborative Innovation Center of Suzhou Nano Science and Technology, the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the 111 Project.

AUTHOR CONTRIBUTIONS
C.W. designed the project. Q.M. and Q.F. contributed equally to this work. Q.M., Q.F., J.X., J.B., X.H., and Z.D. performed the experiments and collected the data. All authors analyzed and interpreted the data, contributed to the writing of the manuscript, discussed the results and implications, and edited the manuscript at all stages.

DECLARATION OF INTERESTS
C.W. and Q.M. have applied for patents related to this study. Z.G. is a scientific co-founder of ZenCapsule Inc. The other authors declare no competing interests.

Received: April 4, 2020
Revised: May 3, 2020
Accepted: May 16, 2020
Published: May 22, 2020

REFERENCES
1. Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature 454, 428–435.
2. Medzhitov, R. (2010). Inflammation 2010: new adventures of an old flame. Cell 140, 771–776.
3. Cazzola, M., Matera, M.G., and Pezzuto, G. (2005). Inflammation— a new therapeutic target in pneumonia. Respiration 72, 117–126.
4. World Health Organization (2020). Coronavirus Disease 2019 (COVID-19): Situation Report-112. https://reliefweb.int/report/world/coronavirus-disease-2019-covid-19-situation-report-112-11-may-2020.
5. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506.
6. Mehta, P., McCullough, D.F., Brown, M., Sanchez, E., Tattersall, R.S., and Manson, J.J.; HLH Across Speciality Collaboration UK (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 395, 1033–1034.
7. Moore, B.J.B., and June, C.H. (2020). Cytokine release syndrome in severe COVID-19. Science 368, eabb9725.
8. Li, H., Liu, L., Zhang, D., Xu, J., Dai, H., Tang, N., Su, X., and Cao, B. (2020). SARS-CoV-2 and viral sepsis: observations and hypotheses. Lancet. https://doi.org/10.1016/S0140-6736(20)30920-X.
9. Cao, X. (2020). COVID-19: immunopathology and its implications for therapy. Nat. Rev. Immunol. https://doi.org/10.1038/s41577-020-0308-3.
10. Schett, G., Sticherling, M., and Neurath, M.F. (2020). COVID-19: risk for cytokine targeting in chronic inflammatory diseases? Nat. Rev. Immunol. https://doi.org/10.1038/s41577-020-0312-7.
11. Kerry, R.G., Sahoo, S.M., Dai, G., and Patra, J.K. (2018). Conventional and nano-based therapy against chronic inflammatory autoimmune diseases. Asian J. Biol. 6, 1–8.
12. Caetano, J., Oliveira, S., and Alves, J.D. (2017). Biological therapy in systemic sclerosis. In Systemic Sclerosis, M. Radic, ed.. https://doi.org/10.5772/intechopen.69326.
13. Rosman, Z., Shoenfeld, Y., and Zandman-Goddard, G. (2013). Biologic therapy for autoimmune diseases: an update. BMC Med. 11, 88–100.
14. Zhao, R., Wang, H., Wang, X., and Feng, F. (2017). Steroid therapy and the risk of osteonecrosis in SARS patients: a dose-response meta-analysis. Osteoporos. Int. 28, 1027–1034.
15. Zhang, S., Ermann, J., Succi, M.D., Zhou, A., Hamilton, M.J., Cao, B., Korzenik, J.R., Glickman, J.N., Vemula, P.K., and Gilmcher, L.H. (2015). An inflammation-targeting hydrogel for local drug delivery in inflammatory bowel disease. Sci. Transl. Med. 7, https://doi.org/10.1126/scitranslmed.aac5657.
16. Zhang, S., Langer, R., and Traverso, G. (2017). Nanoparticulate drug delivery systems targeting inflammation for treatment of inflammatory bowel disease. Nano Today 16, 82–96.
17. Flores, A.M., Hosseini-Nassab, N., Jarz, K.U., Ye, J., Zhu, X., Wirka, R., Koh, A.L., Tsantilas, P., Wang, Y., Nanda, V., et al. (2020). Pro-efferocytic nanoparticles are specifically taken up by lesional macrophages and prevent atherosclerosis. Nat. Nanotechnol. 15, 154–161.
18. Han, X., Wang, C., and Liu, Z. (2018). Red blood cells as smart delivery systems. Bioconjug. Chem. 29, 852–860.
19. Lu, Y., Hu, Q., Jiang, C., and Gu, Z. (2019). Platelet for drug delivery. Curr. Opin. Biotechnol. 58, 81–91.
20. Han, X., Chen, J., Chu, J., Liang, C., Ma, Q., Fan, Q., Liu, Z., and Wang, C. (2019). Platelets as platforms for inhibition of tumor recurrence post-physical therapy by delivery of anti-PD-L1 checkpoint antibody. J. Control. Release 304, 233–241.
21. Wu, G., Zhang, J., Zhao, Q., Zhuang, W., Ding, J., Zhang, C., Gao, H., Pang, D.W., Pu, K., and Xie, H.-Y. (2020). Molecularly engineered macrophage-derived exosomes with inflammation tropism and intrinsic heme biosynthesis for atherosclerosis treatment. Angew. Chem. Int. Ed. 132, 4097–4103.
22. Wen, D., Wang, J., Van Den Driessche, G., Chen, Q., Zhang, Y., Chen, G., Li, H., Soto, J., Liu, M., Shashi, M., et al. (2019). Adipocytes as anticancer drug delivery depot. Matter 1, 1203–1214.
23. Zaldivia, M.T., McFadyen, J.D., Lim, B., Wang, X., and Peter, K. (2017). Platelet-derived microvesicles in cardiovascular diseases. Front. Cardiovasc. Med. 4, 74.
24. Hansson, G.K., Libby, P., and Tabas, I. (2015). Inflammation and plaque vulnerability. J. Intern. Med. 278, 483–493.
25. Song, Y., Huang, Z., Liu, X., Pang, Z., Chen, J., Yang, H., Zhang, N., Cao, Z., Liu, M., and Cao, J. (2019). Platelet membrane-coated nanoparticle-mediated targeting delivery of Rapamycin blocks atherosclerotic plaque development and stabilizes plaque in apolipoprotein E-deficient (ApoE-) mice. Nanomedicine 15, 13–24.
26. Wang, C., Sun, W., Ye, Y., Hu, O., Bomba, H.N., and Gu, Z. (2017). In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy. Nat. Biomed. Eng. 1, 0011.
27. Jung, H., Kang, Y.Y., and Mok, H. (2019). Platelet-derived nanovesicles for hemostasis without release of pro-inflammatory cytokines. Biomater. Sci. 7, 856–859.
28. Sun, S., Zhao, G., Liu, C., Wu, X., Guo, Y., Yu, H., Song, H., Du, L., Jiang, S., and Guo, R. (2013). Inhibition of complement activation alleviates acute lung injury induced by highly pathogenic avian influenza H5N1 virus infection. Am. J. Respir. Cell. Mol. Biol. 49, 221–230.
29. Matuschak, G.M., and Lechner, A.J. (2010). Acute lung injury and the acute respiratory distress syndrome: pathophysiology and treatment. Mo. Med. 107, 252–258.
30. Chu, D., Gao, J., and Wang, Z. (2015). Neutrophil-mediated delivery of therapeutic nanoparticles across blood vessel barrier for treatment of inflammation and infection. ACS Nano 9, 11800–11811.
31. Butt, Y., Kudowska, A., and Allen, T.C. (2016). Acute lung injury: a clinical and molecular review. Arch. Pathol. Lab. Med. 140, 345–350.
32. Aatonen, M., Valkonen, S., Boing, A., Yuana, Y., Niewland, R., and Siljander, P. (2017). Isolation of platelet-derived extracellular vesicles. In Exosomes and Microvesicles: Methods and Protocols, A.F. Hill, ed. (Springer), pp. 177–188.
33. Hu, Q., Sun, W., Wang, J., Ruan, H., Zhang, X., Ye, Y., Shen, S., Wang, C., Lu, W., and Cheng, K. (2018). Conjugation of haematopoietic stem cells and platelets decorated with anti-CD-1 antibodies augments anti-leukaemia efficacy. Nat. Biomed. Eng. 2, 831–840.
34. Nording, H.M., Seizer, P., and Langer, H.F. (2015). Platelets in inflammation and atherogenesis. Front. Immunol. 6, 98.
35. Zhao, G., Wu, H., Jiang, K., Rui, G., Zhu, Z., Qiu, C., Guo, M., and Deng, G. (2016). IFN-γ inhibits S. aureus-induced inflammation by suppressing the activation of NF-κB and MAPks in RAW 264.7 cells and mice with pneumonia. Int. Immunopharmacol. 35, 332–340.
36. Wu, Q., Li, H., Qiu, J., and Feng, H. (2014). Betulin protects mice from bacterial pneumonia and acute lung injury. Microb. Pathogen. 75, 21–28.
37. Chen, P.-Y., Qin, L., Baeyens, N., Li, G., Afolabi, T., Budatha, M., Tellides, G., Schwartz, M.A., and Simons, M. (2015). Endothelial-to-mesenchymal transition drives atherosclerosis progression. J. Clin. Invest. 125, 4514–4528.
38. Liu, Y.J., Sun, D.D., Fan, Q., Ma, Q.L., Dong, Z.L., Tao, W.W., Tao, H.Q., Liu, Z., and Wang, C. (2020). The enhanced permeability and retention effect based nanomedicine at the site of injury. Nano Res. 13, 564–569.
39. Podolin, P.L., Callahan, J.F., Bolognese, B.J., Li, Y.H., Carlson, K., Davis, T.G., Mellor, G.W., Evans, C., and Roshak, A.K. (2009). Attenuation of murine collagen-induced arthritis by a novel, potent, selective small molecule inhibitor of iκB kinase 2, TP-CA-1 (2-(aminocarbonyl) amino)-5-(4-fluorophenyl)-3-thiophenecarboxamide), occurs via reduction of proinflammatory cytokines and antigen-induced T cell proliferation. J. Pharmacol. Exp. Ther. 312, 373–381.
40. Sachse, F., Becker, K., Basel, T., Weiss, D., and Rudack, C.J.R. (2011). IFN-κB inhibitor TP-CA-1 represses nasal epithelial inflammation in vitro. Rhinology 49, 168–173.
41. Zhang, C.Y., Lin, W., Gao, J., Shi, X., Davaritouchane, M., Nielsen, A.E., Mancini, R.J., and Wang, Z. (2019). pH-responsive nanoparticles targeted to lungs for improved therapy of acute lung inflammation/injury. ACS Appl. Mater. Interfaces 11, 16380–16390.
42. Greten, F.R., Arkan, M.C., Bolliath, J., Hsu, L.-C., Goode, J., Methling, C., Gökata, S.N., Neuenhahn, M., Fierer, J., Paxian, S., et al. (2007). NF-κB p105 is a negative regulator of IL-1β secretion as revealed by genetic and pharmacological inhibition of IκB kinase. Cell 130, 918–931.
43. Jaganjac, M., Cipak, A., Schaur, R.J., and Zarkovic, N. (2016). Pathophysiology of neutrophil-mediated extracellular redox reactions. Front. Biosci. 21, 839–855.
44. Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Sun, R., Tian, Z., Xu, X., and Wei, H. (2020). Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. Natl. Sci. Rev. https://doi.org/10.1093/nsr/nwaa041.
45. Huang, X., Xi, H., Zhang, S., and Zhang, G. (2018). The role of macrophages in the pathogenesis of ALI/ARDS. Mediators Inflamm. 2018, 1264913.
46. Byrne, A.J., Maher, T.M., and Lloyd, C.M. (2016). Pulmonary macrophages: a new therapeutic pathway in fibrosing lung disease? Trends Mol. Med. 22, 303–316.
47. Hu, G., and Christman, J.W. (2019). Editorial: alveolar macrophages in lung inflammation and resolution. Front. Immunol. 10, 2275.
48. Wright, S., Ramos, R., Tobias, P., Ulevitch, R., and Mathison, J. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249, 1431–1433.
49. Wieland, C.W., Van Der Wndt, G.J.W., Wiersinga, W.J., Florquin, S., and Van Der Poll, T. (2008). CD14 contributes to pulmonary inflammation and mortality during murine tuberculosis. Immunology 125, 272–279.