Recent advances of natural and bioengineered extracellular vesicles and their application in vascular regeneration

Jianxiong Xu1, Jinxuan Wang1,†, Yidan Chen1, Yuanfang Hou1, Jianjun Hu2,* and Guixue Wang1,*

1Key Laboratory for Biorheological Science and Technology of Ministry of Education, State and Local Joint Engineering Laboratory for Vascular Implants, Bioengineering Modern Life Science Experiment Teaching Center of Bioengineering College, Chongqing University, Chongqing 400030, China
2Department of Pathology, Guizhou Provincial People’s Hospital, Guiyang 550002, China

*Correspondence address. E-mail: wanggx@cqu.edu.cn (G.W.); hujianjun1010@163.com (J.H.)
†These authors contributed equally to this work.

Abstract

The progression of cardiovascular diseases such as atherosclerosis and myocardial infarction leads to serious vascular injury, highlighting the urgent need for targeted regenerative therapy. Extracellular vesicles (EVs) composed of a lipid bilayer containing nuclear and cytosolic materials are relevant to the progression of cardiovascular diseases. Moreover, EVs can deliver bioactive cargo in pathological cardiovascular and regulate the biological function of recipient cells, such as inflammation, proliferation, angiogenesis and polarization. However, because the targeting and bioactivity of natural EVs are subject to several limitations, bioengineered EVs have achieved wide advancements in biomedicine. Bioengineered EVs involve three main ways to acquire including (i) modification of the EVs after isolation; (ii) modification of producer cells before EVs’ isolation; (iii) synthesize EVs using natural or modified cell membranes, and encapsulating drugs or bioactive molecules into EVs. In this review, we first summarize the cardiovascular injury-related disease and describe the role of different cells and EVs in vascular regeneration. We also discuss the application of bioengineered EVs from different producer cells to cardiovascular diseases. Finally, we summarize the surface modification on EVs which can specifically target abnormal cells in injured vascular.

Keywords: cardiovascular disease; extracellular vesicle; vascular regeneration; biomedical engineering

Introduction

Cardiovascular injury is the result of many diseases, such as atherosclerosis, percutaneous coronary intervention, autologous saphenous vein coronary artery bypass grafting and ischemia–reperfusion (I/R). Vascular regeneration or reconstruction after injury is critical for functional recovery. The main problems after vascular injury are endothelial inflammation and thrombosis, which delay the intimal formation and accelerate intimal hyperplasia [1–4]. Conventional clinical treatments are anti-inflammation, anti-thrombus and induction of intima formation [4–6]. Due to the low targeting of drugs and adverse drug reactions such as bleeding events and restenosis, it is difficult for repairing vascular injury with desired effect.

Extracellular vesicles (EVs), which are cell-derived membrane vesicles, are originally discovered to be the mediators of intercellular communication. EVs are natural carriers produced by various cell types, carrying lipids, proteins and RNA [7]. In general, EVs are categorized into four classes: exosomes, 40–100 nm; microvesicles, 100–1000 nm; apoptotic vesicles, more than 800 nm [8, 9]. Meanwhile, the small size of EVs (<200 nm), which can penetrate the endothelial barrier and enter deep tissue, are widely applied in nanomedicine [10]. Studies have shown that natural EVs play an important role in injured vascular regeneration through regulating vascular smooth muscle cells (VSMCs) phenotype transition and endothelial cells (ECs) integrity [11]. However, the complex extraction process and biological function of EVs limit their application in clinical treatment. Bioengineered EVs derived from cell membranes, which have both original and modified functions, are widely used in targeted therapy. In recent years, bioengineered EVs have been used as carriers for nanomaterials, which have advantages in low cytotoxicity, strong targeting and immune escape [9].

In this review, we briefly introduce the molecular mechanism and regenerative process of vascular injury. We summarize the application of natural EVs and bioengineered EVs in the...
treatment of cardiovascular diseases and describe the function of EVs in injured vascular regeneration. Meanwhile, we further compare the characteristics and functions of different EVs and summarize targeted strategies for vascular injury-related diseases. We also introduce bioengineered methods to construct EVs and improve the bioactivity and targeting of EVs. Finally, we discuss the application of bioengineered EVs in the therapy of vascular injury-related diseases and the future of bioengineered EVs.

The molecular mechanism of vascular regeneration after injury

Vascular injury, caused by blood flow shear stress, inflammation and fibrosis, is the pathological foundation and causes of multi-cardiovascular diseases. Meanwhile, vascular injury usually occurs after the surgery of arterial occlusive disease, such as carotid endarterectomy, balloon dilatation surgeries and so on [12, 13]. The regeneration of injured vascular is a complex but orderly process. Generally, there are three stages of vascular repair, including inflammation, neointima formation and vascular remodeling. During the repair process, the first step is platelet aggregation and inflammatory cell infiltration; second, VSMCs will proliferate and ECs migrate to the vascular injury site; finally, the extracellular matrix is deposited in the injured site and then vascular remodeling has been completed (Fig. 1).

ECs and endothelial progenitor cells

ECs, as the interface between the blood and tissues, are important in anti-thrombus, anti-inflammation and vascular barrier function. Importantly, ECs, which play an important role in intracellular communication during vascular injury, are considered as potential mediators of cardiovascular diseases. Furchgott and Zawadzki have first demonstrated that ECs can release endothelium-derived relaxing factors [3], which participate in multi-biological processes, such as oxidative stress, inflammation, vasoconstriction and thrombosis [27, 28].

After intimal injury, the repair of endothelial layer includes two main ways [29]. First, the regenerative cells at injured sites are mostly derived from nearby uninjured cells. Researchers find that the re-endothelialization of arterial intima has three...
independent stages: cleaning, rapid re-endothelialization/proliferation and maturation. This dynamic process starts at 6–24 h after endothelial injury. Then, ECs are able to complete re-endothelialization at 48 h after injury, and maturation when the ECs form continuous and integrity intimal structure at 96 h [30, 31]. Second, the circulating EPCs, including early EPCs and late EPCs, can promote the regenerative process of vascular intima [32, 33]. Early EPCs secrete a variety of angiogenic factors such as vascular endothelial growth factor (VEGF),stromal cell-derived factor 1 and so on, which participate in recruiting mature ECs and regulating ECs proliferation during vascular injury [34–36]. Late EPCs exhibit high cell proliferation rate and strong ability to promote regeneration [37, 38]. Wakabayashi et al. have reported that CD157+ EPCs display regenerative property and are essential for re-endothelialization [39]. Meanwhile, EPC/EC-derived EVs, containing varieties of miRNA and protein, also play a crucial role in attenuating vascular injury and promoting vascular regeneration [40, 41] (Fig. 1). During vascular injury, endothelial-to-mesenchymal transition (EndMT), as a cellular differentiation process, results in migration and fate transition of EC. The EndMT-derived myofibroblasts not only attenuate inflammation but also have the ability to deal with dead cells, which is important for myocardial infarction (MI) recovery [42]. However, excessive EndMT will affect collagen–matrix metalloproteinase balance, fibrosis and correlate with an unstable plaque phenotype in atherosclerosis and in-stent stenosis [43, 44]. Thus, the molecular mechanism of endothelial phenotype transition during vascular injury provides an insight into targeted therapy for re-endothelialization and the treatment of vascular disorders.

**Vascular smooth muscle cells**

VSMCs, as the media parts of vascular wall, are essential for vascular homeostasis. During vascular injury, VSMCs will switch cell fate into migratory and proliferative phenotypes. Therefore, VSMCs can be diverted into ‘contractile’ type (differentiated, non-proliferative) and ‘synthetic’ types (dedifferentiated, proliferative) [45]. The ‘synthetic’ VSMCs have stem cell properties, which exhibit high proliferation and soft stiffness, in response to inflammation and biomechanics during vascular injury [46, 47]. Meanwhile, the unexpected activated VSMCs will migrate from media to intima and start to proliferate after excessive vascular injury [48]. These alterations will result in intimal hyperplasia and lead to vascular stenosis, which significantly increases the risk of cardiovascular diseases, such as MI and stroke. Many growth factors and inflammatory factors, such as transforming growth factor-β, platelet-derived growth factor and interferon, lead to transforming the phenotype of VSMCs and regulating VSMCs proliferation [49]. Furthermore, researchers have also reported that EVs secreted by multiple cell types from pathological microenvironment play an important role in VSMCs phenotype transition and vascular tissue repair [11, 50, 51] (Fig. 1). Thus, EVs loading different contents may effectively target abnormal VSMCs and have potential in the therapy of cardiovascular disease.

**Macrophages**

Macrophages, as the important member of immune cells, can be recruited to the injured sites within 15 min to resolve inflammation and promote tissue repair [52–54]. In vascular injured diseases, such as atherosclerosis, MI and stroke, macrophages are essential for vascular repair and regeneration, contributing to ECs migration and proliferation, neovascularization and SMCs phenotype transition [55–57]. During vascular repair, macrophages will differentiate into pro-inflammatory M1 and anti-inflammatory M2, which is according to the different processes of injury [58]. M1 macrophages can secrete inflammatory cytokines to promote the initial infiltration of circulatory leukocytes [59]. After the early inflammatory phase, macrophages will clean necrotic cell debris via phagocytosis and conversion of M1 to pro-repair and anti-inflammatory M2 phenotype [60]. The M2 macrophages can promote cellular proliferation and vascular remodeling by controlling the production of growth factors, such as VEGF, transforming growth factor β1, insulin-like growth factor 1 and so on [55]. Furthermore, Liu et al. have reported that macrophages also directly mediate vascular repair via using mechanical forces to drag ECs healing and facilitate tight junction [61].

EVs from macrophages also play an important role in regulating vascular microenvironment and various pathophysiological processes. Recent research revealed that macrophages-derived EVs can regulate vascular ECs proliferation, migration and angiogenesis in cardiovascular disease [62] (Fig. 1). Thus, EVs as the regulator of cell/cell communication may act as targeting liposomes for drug delivery and therapy during vascular injury.

### The different derived EVs for vascular therapy

In recent years, nanotechnology has been widely used in the diagnosis and treatment of cardiovascular diseases [63, 64]. However, it is difficult for synthetic nano-drugs to target vascular injured sites through cell biological interactions. EVs are cell-derived membrane vesicles, which are essential for intracellular biological transduction and cellular communication [65]. In vascular injury sites, EVs released from activated vascular cells containing multi-bioactive factors are able to become promising candidates to treat cardiovascular diseases, which have ability in regulating cell proliferation, migration, trans-differentiation and apoptosis (Table 1). Meanwhile, because almost all cells are capable of secreting EVs, EVs are present in various organizations with long-time circulation and can be artificially isolated from body fluids such as blood, urine, cerebrospinal fluid and saliva in large quantities [66]. Therefore, using EVs as the shell of nano-drugs can prolong circulation time and greatly improve delivery efficiency. Moreover, researchers have created bioengineered EVs via modifying EV membranes and loading drugs into EVs to improve bio-targeting and bioactivity (Fig. 2) [67–69]. Furthermore, biomimetic EVs system constructed by cell membrane retains the membrane surface structure and unique biological functions. These systems have obvious advantages for systematic delivery of drugs in cardiovascular diseases, e.g. membrane encapsulating drugs at nanoscale can protect them from immune system and increase drug delivery efficiency with low toxicity [13, 70]. Due to different cell-derived EVs playing distinct roles in the process of vascular regeneration, it is important to select suitable EV systems for the therapy of vascular injured diseases. Here, we summarize the commonly used EVs system in vascular injured diseases including red blood cell (RBC)-derived EVs, EC-derived EVs, macrophage-derived EVs, platelet-derived EVs and stem cell-derived EVs (Table 1).

### RBC-based EV system

RBCs (also as erythrocyte), as the most abundant type of blood cells, play an important role in transporting oxygen and nutrients [85]. Meanwhile, RBCs not only have long-term circulatory property, but also RBCs-derived microvesicles can regulate a variety...
| Donor cell | Methods | Animal model | Administration | Characteristics | Effects | References |
|------------|---------|--------------|---------------|----------------|---------|------------|
| RBC        | • Extrusion through 200 nm ppm | Mouse atherosclerosis | i.v. | • Immune escape | • Decrease the size of atherosclerotic plaque | [71] |
|            | • Loading with PLGA-rapamycin NPs | | | • Long circulating time | • Reduces inflammation | |
|            | • Probe sonication | Mouse I/R | i.v. | | | |
|            | • Modification with stroke homing peptide | | | | | |
|            | • Loading with PHB-dextran-NR2B9C NPs | | | | | |
|            | • Probe sonication | Mouse thrombosis | i.v. | | | |
|            | • Modifying with fibrin-targeting peptide | | | | | |
|            | • loading with dextran–tirofiban conjugate NPs | | | | | |
| EC         | • Centrifugation at 20 500 g | Mouse atherosclerosis | i.v. | • miR-143/145 enrichment | • Decrease the size of atherosclerotic plaque | [21] |
|            | • Overexpression Klf2 in donor cells | Mouse I/R | i.v. | • miR-24-3p enrichment | • Transits SMC to athero-protective phenotype | |
|            | • Gradient centrifugation | | | | | |
|            | • Overexpression Cxcr4 in donor cells | Mouse I/R | i.v. | • Targeting Sdf-1 high expressed area | • Decrease I/R injury | [15] |
|            | • Extrusion through 200 nm ppm | | | • ROS-responsive drug release | • Reduces the recruitment of Ly6C⁺ monocyte | |
|            | • Loading with HOP conjunct rapamycin NPs | | | • high biocompatibility | | |
| Macrophage | • IL-4 stimulates donor cell | Mouse atherosclerosis | i.p. | • miRNA-99a/146b/378a enrichment | • Decreases inflammation and necrotic lesion areas | [23] |
|            | • Centrifugation at 100 000 g | | | • Using M2 macrophage-derived exosomes | • Accelerates vascular tissue repair | [11] |
|            | • Centrifugation at 100 000 g | Rat stent implantation | Local delivery | | • Promotes VSMC dedifferentiation | |
|            | • Centrifugation at 100 000 g | Mouse atherosclerosis | i.p. | | • Increases the anti-inflammatory effects | [75] |
|            | • Loading with HAL by electroporation | | | | • Alleviates atherosclerosis | |
|            | • IL-1β, IL-6R and TNF-αR plasmids are transfected in donor cells | Mouse MI | i.v. | • Targeting chemokine-enriched area | • Accelerates the recovery of cardiac function | [76] |
|            | • Extrusion through 400 nm ppm | | | | • Prevents hypoxia-induced apoptosis | |
|            | • Loading with miR-199a-3p-PEG-PLA NPs | | | • Targeting IL-1β, IL-1β, TNF-α enriched area | | |
|            | • Extrusion and sonication | Mouse atherosclerosis | i.v. | • miR-199a-3p enrichment | • Increases anti-thrombotic activity | [73] |
|            | • Loading with Oxi-COS-atorvastatin NPs | | | • ROS-responsive drug release | | |

(continued)
| Donor cell          | Methods                                      | Animal model            | Administration | Characteristics                                      | Effects                                      | References |
|---------------------|---------------------------------------------|-------------------------|----------------|-----------------------------------------------------|---------------------------------------------|-----------|
| Platelet            | • Centrifugation at 100 000 g               | Rat MI                  | Left ventricle | • Stimulates VEGF, bFGF signaling pathway            | • Improve the process of revascularization | [78]      |
|                     | • Probe sonication                          |                         |                | • Collagen binding (injured target)                  | • Suppresses coronary restenosis            | [79]      |
|                     | • Loading with docetaxel–PLA NPs            | Balloon vascular injury | i.v.           | • Immuno-compatibility                               |                                             |           |
|                     | • Modifying with PEG on donor cell          | Mouse atherosclerosis   | i.v.           | • Accumulation in atherosclerotic plaque              | • Attenuates the progression of atherosclerosis | [80]      |
|                     | • Probe sonication                          |                         |                |                                                     |                                             |           |
|                     | • Loading with PLGA–rapamycin NPs           |                         |                |                                                     |                                             |           |
| Progenitor/stem cell| • Centrifugation at 100 000 g               | Rat stent implantation  | Coating on stent | • Stem cell-derived EVs have pro-healing property    | • Accelerates re-endothelialization        | [81]      |
|                     | • Overexpression Gata4 in donor cell        | Rat MI                  | i.o.           | • Enrich anti-apoptotic miRNAs (miR-19a)            | • Restores cardiac contractile function    | [82]      |
|                     | • 0.2μm filtration and gradient certification|                         |                |                                                     | • Reduces infarct size                      |           |
|                     | • Overexpression Cxcr4 in donor cell        | Mouse hindlimb ischemic | i.v.           | • Targeting ischemic tissue                         | • Enhances blood reperfusion               | [83]      |
|                     | • Probe sonication                          |                         |                |                                                     | • Accelerates limb salvage                 |           |
|                     | • Loading with VEGF–PLGA NPs by sonication  |                         |                |                                                     |                                             |           |
|                     | • Loading iron oxide NPs in donor cell      | Rat I/R                 | i.v.           | • Therapeutic growth factors                         | • Decreases infarction volume              | [84]      |
|                     | • Extrusion through 400 nm ppm              |                         |                | • Magnetically guided, targeted drug delivery        | • Promotes angiogenesis, anti-apoptosis and anti-inflammation |           |

ppm, polycarbonate porous membrane; PEG–PLA, poly (ethylene glycol–poly(lactic acid); PLGA, poly (lactic-co-glycolic acid); HCF, p-hydroxybenzyl alcohol-oxyal chloride-poly (ethylene glycol); Oxi-COS, amphiphilic oxidation-sensitive chitosan oligosaccharide; HAL, hexyl 5-aminolevulinate hydrochloride; i.v., intravenous injection; i.p., intraperitoneal injection; i.o., intramyocardial injection; local delivery, drugs were preloaded into pluronic gel F-127 (Sigma) and locally dress around the injured artery; I/R, ischemia-reperfusion; MI, myocardial infarction, NPs, nanoparticles.
of cardiovascular diseases [86]. These long-circulating, biocompatible and non-immunogenic properties enable RBCs and their derived EVs to serve as protective shells for cargoes [87]. Recently, Usman et al. constructed a RBCs-derived EVs delivery system to load miRNA and CRISPR–Cas9 system showing higher transfecting efficiency than traditional reagents [88]. Furthermore, RBC membrane also has self-immune escape marker Cd47 and polysaccharide coating, which are essential for cell stability and immune escape [89]. Thus, RBCs membrane can be utilized to package drugs or small molecular, such as polymeric NPs and other nano-drug to construct biomimetic EVs with extended circulation time up to 72 h [90]. Our lab recent work uses RBCs encapsulating core-shell structured nanocomplexes to target atherosclerosis. Animal experimental results show that

Figure 2. The synthetic strategies of bioengineered EVs. Producer cells can be loaded with therapeutic molecules such as drugs, nanoparticles (NPs), nucleic acid species and proteins via incubation with cells, lipofection and electroporation. Producer cells also can be transfected with plasmid or mRNA via lipofection, electroporation and viral transfection to express protein and peptides which have therapeutic and targeting characteristics. EVs from producer cells are then isolated through centrifugation with different centrifugal force. Moreover, EVs also can be synthesized by membrane extrusion and probe sonication. The bioengineered cell membrane fragments are extruded through pore size 0.1–1 μm polycarbonate film or sonicated in a water bath to synthesize biomimetic EVs. Biomimetic EVs have the biological inherencies from the producer cells including specifically surface receptor and bioactive molecular. Moreover, the drugs can be loaded in biomimetic EVs during synthesis.
RBC-derived biomimetic EVs reduce immune clearance and increase the accumulation of NPs in atherosclerotic plaque [71]. Moreover, due to its highly flexible structure, RBC-derived biomimetic EVs can load different core NPs including polymeric NPs [91], magnetic NPs [92], gold NPs [93] and so on. These excellent properties make RBCs-based biomimetic EVs system have great potential in the treatment of cardiovascular diseases (Table 1).

**EC-based EV system**

ECs, as the functional regulator in vascular homeostasis, especially in barrier function, inflammation and thrombus. Recent study has reported that EC-derived small EVs (sEVs) act as a new link between ECs and SMCs in vascular and play an important role in angiogenesis and the transition of SMC phenotype [94]. EVs containing miR-143/145 that are isolated from Kif2-overexpressing ECs can regulate SMC phenotypes and reduce atherosclerotic lesion formation [21]. Moreover, these KLF2-overexpressing EC-derived EVs also can attenuate myocardial I/R injury and reduce the recruitment of Ly6C+ monocyte [15]. Due to EC-derived EVs having great potential in cardiovascular disease, our lab recent work uses CXC4R-overexpressing primary mouse thoracic aorta EC-derived EVs loading with HBA-PC-PEG conjunct rapamycin NPs to target SDF1 highly expressed and ROS accumulated area during cerebral I/R injury. These synthetic EVs significantly improve cerebral I/R injury and suppress local inflammation [74]. Thus ECs-based EVs have been widely applied in the treatment of cardiovascular diseases.

**Macrophage/monocyte-based EV system**

Macrophage, which has specific receptors to recognize inflammation, tissue debris and foreign invasion, is the ‘guardian’ of the body [95]. Because EVs can inherit the membrane functional receptors from donor cells, macrophage-derived EVs are widely applied in injury and inflammation-related diseases [96, 97]. Macrophage-derived EVs show perfect inflammation-tropism and anti-inflammation properties via high expression of chemokine receptors and releasing anti-inflammatory cytokines [98]. Moreover, because M2 macrophage and its EV play an important role in targeting and resolving inflammation and tissue remodeling, researchers have utilized M2 macrophage-derived EVs to promote vascular regeneration. Recently, researchers have reported that bioengineered M2 macrophages exosomes loading with hexyl S-aminovulinate hydrochloride via electroporation exhibit excellent abilities for targeting chemokine-enriched area and reducing vascular necrotic area in atherosclerosis [75]. Furthermore, because EVs can involve in cell-to-cell communication, they contain numerous cargoes including miRNAs, lncRNAs, proteins and lipids that may act as therapeutic agents in cardiovascular diseases (Fig. 1) [99]. Bouchareychas et al. utilize IL-4 to foster macrophages to M2 polarization and find that these macrophage-derived exosomes, which contain miRNA-99a/146b/378a, can reduce inflammation and atherosclerotic plaque in vascular injury [23]. Because macrophages can adhere on vascular injured site, macrophage membrane is utilized to encapsulate ROS-responsive PCM-rapamycin NPs for targeting ROS accumulated and inflammatory area in pathological vascular injury. These biomimetic EVs can effectively inhibit intimal hyperplasia with low cytotoxicity on ECs [97, 100]. Moreover, Martinez et al. have utilized macrophage membrane proteins hybridizing with liposomes to target inflammatory ECs [101]. Because macrophage-based EV can target inflammatory site, which exhibits superior accumulation in injured area and can load a wide variety of solubility nano-drugs and contrast agents, they are increasingly used in all aspects of vascular injury-related disease therapy (Table 1).

**Platelet-based EV system**

Platelets are non-nucleated blood cells produced by mature megakaryocytes in the bone marrow and lungs, which play an important role in the process of coagulation, hemostasis, inflammation and immune regulation [102, 103]. Platelet-derived EVs contain nucleic acids, growth factors, lipids and proteins, can regulate injury repair, inflammation and immune response after vascular injury [104]. Brill et al. have reported that platelet-derived EVs can stimulate angiogenesis through VEGF signaling after MI [78]. Moreover, EVs derived from thrombin/collagen-induced platelets can enhance the adhesion of early outgrowth cells in vascular injured sites and promote re-endothelialization [105]. Meanwhile, due to platelets having the ability to adhere and aggregate in injured site, Hu et al. prepare biomimetic EVs by encapsulating docetaxel-loaded poly(lactic-co-glycolic acid) (PLGA) NPs in platelet membrane. These biomimetic EVs have great immunocompatibility and collagen targeting, which can selectively adhere to injured vascular and efficiently suppress coronary restenosis [79]. Moreover, to improve the circulation time of EVs in blood, PEG was utilized to modify on platelet membrane. These PEG-modified platelet-derived EVs loading with PLGA-rapamycin NPs exhibit 4.98-fold accumulated efficiency than normal NP in atherosclerotic arterial trees and significantly attenuate the progression of atherosclerosis [80]. Platelet-derived EVs preferentially accumulate in the collagen exposure area, which is associated with intimal injury, demonstrating an effectively targeted ability to vascular injured sites.

**Progenitor/stem cell-based EV system**

Stem/progenitor cells are self-renewing, multipotent cells that reside in various tissues and play an important role in tissue repair, anti-inflammation and multilineage differentiation [106, 107]. Mesenchymal stem cell (MSC) as the abundant stem cell in vascular, which can produce amounts of EVs during physiological environment, is essential for tissue repair and differentiating to replace injured cells [108]. Meanwhile, a large amount of miRNA has been identified in MSC-derived EVs, many of which can inhibit inflammation and promote neovascularization [82]. Thus, recent studies have used MSC-derived exosomes to treat cardiovascular injury. Hu et al. fabricate MSC-derived exosome-released stent that can promote vascular healing and accelerate re-endothelialization after stent implantation [81]. Moreover, Zhao et al. find that MSC-derived exosomes can restore cardiac contractile function and transform macrophages to M2 phenotype through miR-182 [109]. Furthermore, peptide GSPRETSYMPH is used to cross-link with MSC-derived nanovesicles, which can target disturbed flow site and significantly contribute to endothelial recovery after injury [110]. Due to the targeting ability of natural EVs still needing to enhance, CXC4R is overexpressed in stem cells through plasmid transfection to acquire robust targeting. Then PLGA–VEGF NPs are encapsulated by cell membrane to construct nanovesicles, which have great potential for delivering VEGF to injured sites and promoting the recovery of vascular function [83]. Furthermore, Kim et al. co-cultured MSCs with iron oxide NPs to stimulate the expression of therapeutic growth factors in MSC and made MSC-derived EVs obtain magnetic targeting. The magnetic navigation makes MSC-derived EVs localize on the ischemic lesion and have great effects on the treatment of...
ischemic stroke [84]. Stem cell-derived EVs have great potential in the treatment of vascular injury due to their regenerative cargos.

Hybrid membrane-based biomimetic EV system

Cell membrane-wrapped biomimetic EV, which can be simply fabricated, is a new targeted nano-drug delivery system. Although single-type cell membrane-based EVs system has been applied in cardiovascular disease therapy, it is still struggle to have therapeutic efficacy in the pathological microenvironment after vascular injury. Thus, recent researchers develop hybrid RBC–EC membrane-cloaked NPs to target choroidal neovascularization, in which RBC membrane is used to reduce immune phagocytosis and EC membrane is used as anti-VEGF nano agents to target retinal endothelialcytes [111]. Moreover, because RBCs have long-term circulatory property, RBC–platelet hybrid and RBC–cancer membrane hybrid are used to target tumor area and prolong drug circulation time [112–114]. Meanwhile, due to the platelet membrane also having immune-evading and cancer cell-binding ability, platelet membranes are widely used to fuse with other cell membranes such as leukocyte and cancer stem cell [115–117]. Furthermore, platelet membrane also is used to fuse with stem cell exosomes to enhance exosomes’ capability to target injured vascular [118]. Combination of the differently biological membrane can obtain both functions and characteristics of producer cells, which improve the targeting ability and circulating time.

The bioengineered strategies for EVs to target vascular injured site

EVs have been considered as advanced strategies to treat vascular injured disease, whereas, systemic exposure and off-target effect have limited the development of cardiovascular therapies. Moreover, the repair of vascular injury, as a multiple physiological process, is regulated by a variety of cells and factors, which make drugs hardly directly to the diseased site and have therapeutic index. For example, after stent implanting into the carotid arteries, preventing neointimal hyperplasia and re-endothelialization are the most important events to avoid arterial re-obstruction. Although anti-proliferative drugs such as rapamycin or paclitaxel have great effect on preventing neointimal thickening, the re-endothelialization also will be delayed by the suppression of endothelial proliferation. Meanwhile, compared with paclitaxel-eluting stents, MSC-derived exosomes-eluting stent accelerates re-endothelialization, but also promotes SMC proliferation in vascular injured sites [81]. Thus, it is important to modify EVs with targeting proteins or peptides that can specifically bind with cellular receptors and extracellular components expressed in injured vascular and make EVs-based systems can efficiently deliver drugs to targeted sites or even directly to the diseased/abnormal cells. Here, we summarize the targeting peptides and proteins for cardiovascular diseases (Table 2).

Target ECs in vascular injured sites

Vascular intima, which is composed of a continuous ECs layer, maintains the physiological homeostasis of blood vessels [131]. During cardiovascular events, activated ECs can undergo a dramatic transition in their functional phenotype in response to injury and inflammation [132, 133]. Furthermore, inflammatory ECs highly express many adhesion molecules (e.g. ICAM1, VCAM1, E-selectin and P-selectin), which play an important role in immune recruitment [134, 135]. Thus, designing drugs based on these inflammatory biomarkers of ECs can enhance the likelihood of EC targeting and uptake by activated ECs (Table 2). Our lab’s recent study uses integrin αβ1 highly expressed macrophage membrane to encapsulate rapamycin for inhibiting cell inflammation and autophagy. These biomimetic EVs can significantly target activated ECs (VCAM1+) and efficiently reduce inflammation and atherosclerotic plaque [70]. Moreover, M1 as coronary acute ischemia and hypoxia-induced injury needs therapeutic angiogenesis to restore the blood supply. Thus, recent study has used cardiac homing peptide conjugated with cardiac stem cell-derived exosomes to target infarcted heart for regenerative therapy. These targeting exosomes can promote EC proliferation, which contributes to promoting angiogenesis and reducing scar size in heart [136].

Target macrophages/monocytes in vascular injured sites

Macrophages/monocytes, as an important member of immune system, can be recruited to the injured site as soon as 15 min [54], and differentiate into pro-inflammatory M1 and anti-inflammatory M2 macrophages according to the injured degree and type of the injured location [137], whereas uncontrollable M1 macrophages in cardiovascular will exacerbate a variety of inflammation-based disorders, such as atherosclerosis, MI, intimal hyperplasia and so on [11, 18]. During myocardial I/R or other vascular injured diseases, abnormally accumulating M1 macrophages will result in inflammation and a disturbing reparative stage. Therefore, modulation of macrophage polarization is an important method for myocardial and vascular repair. Recent research has used MSC-derived EVs fusing with platelet membrane to mimic the binding effect of platelet CD62p to macrophage Psg1. These modified EVs successfully reprogram M1 macrophages to M2 macrophages and accelerate cardiac repair [138]. Moreover, macrophage polarization markers such as M1 marker: MARCO, HLA-DPB1, CD80, CD86; M2 marker: MRC1, CD163, CD209, CLEC7A also can be used to design macrophages’ targeting EVs [139]. Macrophages that engulf excessive lipids and apoptotic cells via scavenger receptors (CD36, MSR1 and LOX-1) will form foam cells in vascular, resulting in tissue inflammation and apoptosis [140]. Nie et al. have used KODIA-PC-modified liposome-like vesicles to target macrophage CD36 receptor and demonstrate that vesicles can co-localize with CD36 macrophages and accumulation in vulnerable and inflammatory atherosclerotic plaque [125].

Target VSMCs/cardiomyocytes in vascular injured sites

VSMCs are the main cell types involved in constituting arterial vascular wall and ensuring vascular tension. The disorder of VSMCs will trans-differentiate to multiple cells including osteoblasts-like VSMC (Runx2, Mx2), macrophage-like VSMCs (Mac2, Cd11b) and synthetic VSMCs with proliferative property, which contribute to the progression of cardiovascular diseases such as hypertension, atherosclerosis and so on [141]. Thus, recent research mainly focuses on inhibiting the ‘malignant’ proliferation and trans-differentiation of VSMCs in the neo-intima during vascular injury. Wang et al. have used CAR peptide to modify MSC-derived EVs for targeted pulmonary hypertension therapy. CAR-modified EVs significantly target abnormal SMC and inhibit hypoxia-induced proliferation, migration and phenotype transition of SMC during pulmonary hypertension [127]. Moreover, Because PDGFβR is overexpressed in proliferative SMC, PDGF–BB peptide conjugate dexamethasone–PLGA NP is designed
to target injured SMCs, exhibiting significant inhibition of SMCs’ proliferation [126].

Cardiomyocytes as part of muscle cells play an important role in cardiac function. During myocardial I/R injury and infarction, cardiomyocytes as the main stimulus for the inflammation result in excessive necrosis and cardiac dysfunction. Thus, recent researchers have used myocardium-targeting peptides to modify stem cell-derived exosomes for targeting injured cardiomyocytes, which significantly reduce cardiomyocytes necrosis and infarct area, and have improved cardiac function [128, 129].

Discussion

EVs, as secreted plasma membrane cargo carriers, are released from diversities of cells into blood flow and substantive organization under both developmental and stress environments [142]. In the past decades, a lot of work have indicated the physiological functions of EVs, which are essential for intercellular communication and the transduction of biological signals [143]. Cardiovascular diseases, which are accompanied by many risk factors, have complex disease processes and pathogenesis. EVs from vascular injured sites, due to their effect on inflammation, thrombosis, angiogenesis and endothelial homeostasis, play an important role in the initiation and progression of vascular regeneration [144–146]. Meanwhile, recent researchers have reported that exosomes derived from MSCs, as stent coating materials, can accelerate re-endothelialization and promote vascular repair after stent implantation [81, 147], whereas there are still three main limitations in EVs’ drug delivery system: (i) the isolation and purification of EVs especially in <100 nm exosomes currently need ultra-high-speed centrifuge with >100 000 g centrifugal force; (ii) given that the content of EV is determined by its donor cell or tissue fluid, the heterogeneity of donor cell is reflected in the EV, which needs bioengineered methods to reduce this heterogeneity; and (iii) the natural EVs, as the cellular membrane leaflet, have low functional proteins to recognize abnormal cells during targeted therapy.

To improve the EV-drug delivery system, researchers utilize a variety of bioengineered methods to modify natural EVs [148]. Recent efforts rationally provide a contemporary design for EVs-secreting cells to increase targeting components of EVs via stimulating induction and biogenetic modulation (Fig. 2). EVs derived

Table 2. Potential strategies for targeting abnormal cells in cardiovascular diseases

| Targeting cell | Targeting point | Disease | Targeting agent/ peptide | Effects | References |
|----------------|-----------------|---------|---------------------------|---------|------------|
| EC             | VCAM1           | Atherosclerosis | VHPK (peptide) | Target inflammatory EC and reduce atherosclerotic plaque | [119] |
|                | Atherosclerosis | Integrin αβ1 (protein) | | Target activated EC and reduce atherosclerotic plaque | [70] |
| E-selectin     | Atherosclerosis | HPMA (polymer) | | Target inflammatory EC and reduce vascular inflammation | [120] |
| α-2Ars         | Atherosclerosis | Cys-L9R-Cys (peptide) | | Target lipid-activated EC and enhance eNOS expression | [121] |
| Macrophage     | CCR2            | Atherosclerosis | YNFTNRKISVQLAS-YRRITSSK (peptide) | Target monocyte with inflammatory response imaging atherosclerotic area | [122] |
| CCR5           | Vascular injury | DAPTA (peptide) | | Target recruited monocytes and imaging vascular injury | [123] |
| P32            | Atherosclerosis | LYP-1 (peptide) | | Target macrophages in atherosclerotic plaque | [124] |
| CD36           | Atherosclerosis | KOdiA-PC (lipid) | | Target macrophages in atherosclerotic plaque | [125] |
| SMC            | PDGFRβ          | Vascular injury | PDGF-BB peptide | Reduce restenosis and neointimal hyperplasia. | [126] |
|                | Heparan sulfate | Hypertension | CAR (peptide) | Inhibit SMC proliferation and migration | [127] |
| Cardiomyocytes | Unknown         | MI       | CSTSMLKAC (peptide) | Reduce cardiomyocytes necrosis and infarct area | [128, 129] |
| Platelet       | GPIb-IIIa       | Vascular injury | RGD | Imaging the aggravation of platelets. | [130] |

HPMA, N-(2-hydroxypropyl)methacrylamide; LCCA, ligated left common carotid arteries; MI, myocardial infarction.
from stem cells during inducing conditions (such as inflammation [149, 150], hypoxia [151] and so on) can improve vascular function and reduce unexpected inflammation after injury. Furthermore, EVs also can be pre-loaded with exogenous compounds (such as miRNA [152], lncRNA [153] and cytokines [154]) to enhance the bioactivity of EVs. Meanwhile, recent study has found that overexpression of glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase in donor cells can increase the loading efficiency of siRNA and the generative rate of EVs [155]. However, compared with recent nanotechnology, the therapeutic effect and targeting accuracy of natural EVs still need to be enhanced.

Nowadays, biomimetic sEVs derived from synthetic cell membranes are widely utilized for targeted drug delivery, which has high biocompatibility, prolonger circulating time as well as specifically targeted ability. Recent research has created newly mimic exosomes, which combine synthetic liposome and macrophage-derived EVs to enhance drug delivery efficiency [156]. Furthermore, our recent researches find that synthetic RBC-derived sEVs can prolong circulating time, significantly attenuate the progression of atherosclerosis [71] and specifically be enriched in low shear stress area [157]. Moreover, synthetic EVs can also be modified to construct targeting structures on the membrane surface via genetic engineering and chemical conjugation [158, 159].

Overall, EVs as cell-based therapies for vascular injury take unique advantages of surviving in the circulation, breaking through the vascular barrier and delivering molecular cargo to recipient cells. Moreover, EVs can be modulated and synthesized through bioengineered methods, which can easily enhance the bioactivity and targeting of EVs. Meanwhile, during vascular injury, EVs as biological agents derived from repairing cells play an important mediator in the pathophysiological process. Bioengineered EVs loading with single or multiple biomolecules and drugs are emerging as therapeutic methods for vascular regeneration.

Acknowledgements
The authors would like to thank all other members of the Professor Guixue Wang’s laboratory for their constructive discussions as well as the support from the Public Experiment Center of State Bioindustrial Base (Chongqing), China.

Authors’ contributions
G.W., J.W. and J.X. conceived the idea. J.X. and J.W. performed original draft preparation. J.W., J.X., J.H. drafted the picture and table. G.W., J.H. and J.X. wrote and revised the article. G.W. made final approval of the article.

Funding
The work was supported by the National Natural Science Foundation of China [Grant Nos. 12032007, 31971242] to G.W.; Chongqing Science and Technology Bureau [Grant No. cstc2019jcyj-zdxmX0028] to G.W.; Chongqing Municipal Education Commission, China [Grant No. KY2020001] to G.W.; Guizhou Provincial Science and Technology Projects [Grant No. Qiankehejichu(2018)1103] to J.H.

Conflicts of interest statement. All authors state that they have no conflicts of interest.

References
1. Gaudino M, Antoniades C, Benedetto U, Deb S, Di Franco A, Di Giannmarco G, Frenos S, Gineur D, Grau J, He GW, Marinelli D, Ohnnes LB, Patrone C, Puskas J, Tranbaugh R, Girardi LN, Taggart DP, Alliance A; ATLANTIC (Arterial Grafting International Consortium) Alliance. Mechanisms, consequences, and prevention of coronary graft failure. Circulation 2017;136:1749–64.
2. Chaabane C, Otsuka F, Virmani R, Bochaton-Piallat ML. Biological responses in stented arteries. Cardiovasc Res 2013;99:353–63.
3. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–6.
4. Brevetti G, Giugliano G, Brevetti L, Hiatt WR. Inflammation in peripheral artery disease. Circulation 2010;122:1862–75.
5. Adhyaru BB, Jacobson TA. Safety and efficacy of statin therapy. Nat Rev Cardiol 2018;15:757–69.
6. Makaryus JN, Halperin JL, Lau JF. Oral anticoagulants in the management of venous thromboembolism. Nat Rev Cardiol 2013;10:397–409.
7. Antimisiaris SG, Mourtas S, Marazziotis A. Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceuticals 2018;10:218.
8. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009;9:581–93.
9. van der Meel J, Fens MH, Vadar P, van Solinge WW, Eniola-Adefeso O, Schifferlser M. Extracellular vesicles as drug delivery systems: lessons from the inositol phosphate field. J Control Release 2014;195:752–85.
10. Matsumoto J, Stewart T, Banks WA, Zhang J. The transport mechanism of extracellular vesicles at the blood–brain barrier. Curr Pharm Des 2017;23:6206–14.
11. Yan W, Li T, Yin T, Hou Z, Qu K, Wang N, Durkan C, Dong L, Qiu J, Gregersen H, Wang G. M2 macrophage-derived exosomes promote the c-KIT phenotype of vascular smooth muscle cells during vascular tissue repair after intravascular stent implantation. Theranostics 2020;10:10712–28.
12. Zhang Y, Chen S, Zhang H, Ma C, Du T, Qiao A. Model construction and numerical simulation of arterial remodeling after stent implantation with variations of cell concentration. Med Eng Technol Devices 2022;16:100144.
13. Wang H, Xing M, Deng W, Qian M, Wang F, Wang X, Midgley AC, Zhao Q. Anti-Sca-1 antibody-functionalized vascular grafts improve vascular regeneration via selective capture of endogenous vascular stem/progenitor cells. Bioact Mater 2022;16:433–50.
14. Zhang L, Wei Q, Liu X, Zhang T, Wang S, Zhou L, Zou L, Fan F, Chi H, Sun J, Wang D. Exosomal microRNA-98-5p from hypoxic bone marrow mesenchymal stem cells inhibits myocardial ischemia–reperfusion injury by reducing TLR4 and activating the PI3K/Akt signaling pathway. Int Immunopharmacol 2021;101:107592.
15. Qiao S, Zhang W, Yin Y, Wei Z, Chen F, Zhao J, Sun X, Mu D, Xie J, Xu B. Extracellular vesicles derived from Kruppel-Like factor 2-overexpressing endothelial cells attenuate myocardial ischemia–reperfusion injury by preventing Ly6C(high) monocyte recruitment. Theranostics 2020;10:11562–79.
16. Wang J, Chen S, Zhang W, Chen Y, Bihl JC. Exosomes from miRNA-126-modified endothelial progenitor cells alleviate brain injury and promote functional recovery after stroke. CNS Neurosci Ther 2020;26:1255–65.
17. Hu H, Wang B, Jiang C, Li R, Zhao J. Endothelial progenitor cell-derived exosomes facilitate vascular endothelial cell repair through shuttling miR-21-5p to modulate Thrombospondin-1 expression. Clin Sci (Lond) 2019;133:1629–44.

18. Liu S, Chen J, Shi J, Zhou W, Wang L, Fang W, Zhong Y, Chen X, Chen Y, Sabri A, Liu S. M1-like macrophage-derived exosomes suppress angiogenesis and exacerbate cardiac dysfunction in a myocardial infarction microenvironment. Basic Res Cardiol 2020;115:22.

19. Yang Y, Guo Z, Chen W, Wang X, Cao M, Han X, Zhang K, Teng B, Cao J, Wu W, Cao P, Huang C, Qu Z. M2 macrophage-derived exosomes promote angiogenesis and growth of pancreatic ductal adenocarcinoma by targeting EZF2. Mol Ther 2021;29:1226–38.

20. Wang Z, Zhao Y, Zhao Y, Zhang Y, Yao X, Hang R. Exosomes secreted by macrophages upon copper ion stimulation can promote angiogenesis. Mater Sci Eng C Mater Biol Appl 2021;123:111981.

21. Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJ, Zehir AM, Scheffer MF, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmelser S. Atherosprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol 2012;14:249–56.

22. Lin X, Li S, Wang YJ, Wang Y, Zhong JY, He JY, Cui XJ, Zhan JK, Liu YS. Exosomal Notch3 from high glucose-stimulated endothelial cells regulates vascular smooth muscle cells calcification/aging. Life Sci 2019;232:116582.

23. Bouchareychas L, Duong P, Covarrubias S, Alsop E, Phu TA, Nguyen MA, Karunakaran D, Geoffrion M, Cheng HS, Tandoc M, Godo S, Shimokawa H. Endothelial functions. Arterioscler Thromb Vasc Biol 2018;38:49–63.

24. Zhu J, Liu B, Wang Z, Wang D, Ni H, Zhang L, Wang Y. Exosomes from nicotine-stimulated macrophages accelerate atherosclerosis through miR-21-3p/PTEN-mediated VSMC migration and proliferation. Theranosics 2019;9:6901–19.

25. Wang Z, Zhu H, Shi H, Zhao H, Gao R, Weng X, Liu R, Li X, Zou Y, Hu K, Sun A, Ge J. Exosomes derived from M1 macrophages aggravate neointimal hyperplasia following carotid artery injuries in mice through miR-222/CDKN1B/CDKN1C pathway. Cell Death Dis 2019;10:422.

26. Godo S, Shimokawa H. Endothelial functions. Arterioscler Thromb Vasc Biol 2017;37:e108–e114.

27. Muñoz M, López-Oliva ME, Rodríguez C, Martínez MP, Sáenz-Medina J, Sánchez A, Climent B, Benedito S, García-Sacristán A, Rivera L, Hernández M, Prieto D. Differential contribution of Nox1, Nox2 and Nox4 to kidney vascular oxidative stress and endothelial dysfunction in obesity. Redox Biol 2020;28:101330.

28. Heusch G, Libby P, Gersh B, Yellon D, Bohn M, Lopaschuk G, Opie L. Cardiovascular remodelling in coronary artery disease and heart failure. Lancet 2014;383:1933–43.

29. McDonald AI, Shirali AS, Aragon R, Ma F, Hernandez G, Vaughn DA, Mack JJ, Lim TY, Sunshine H, Zhao P, Kalincikenthin V, Hai T, Pelegri M, Ardehali R, Iruela-Arispe ML. Endothelial regeneration of large vessels is a biphasic process driven by local cells with distinct proliferative capacities. Cell Stem Cell 2018;23:210–225.e6.

30. Itoh Y, Toriumi H, Yamada S, Hoshino H, Suzuki N. Resident endothelial cells surrounding damaged arterial endothelium reendothelialize the lesion. Arterioscler Thromb Vasc Biol 2010;30:1725–32.

31. Zampetaki A, Kirtton JP, Xu Q. Vascular repair by endothelial progenitor cells. Cardiovasc Res 2008;78:413–21.

32. Wang M, Xu H, Li Y, Cao Z, Zhu H, Wang Y, Zhao Z, Pei G, Zhu F, Yang Q, Deng X, Zhou C, Guo Y, Wu J, Liao W, Yang J, Yao Y, Zeng R. Exogenous bone marrow derived-putative endothelial progenitor cells attenuate ischemia reperfusion-induced vascular injury and renal fibrosis in mice dependent on pericytes. Theranostics 2020;10:12144–57.

33. Yue Y, Wang C, Benedict C, Huang G, Truongcao M, Roy R, Cimini M, Ganikapiti VNS, Cheng Z, Koj WJ, Kishore R. Interleukin-10 deficiency alters endothelial progenitor cell-derived exosome reparative effect on myocardial repair via integrin-linked kinase enrichment. Circ Res 2020;126:315–29.

34. Bianconi V, Sahebkar A, Kovanen P, Bagaglia F, Ricciuti B, Calabro P, Patti G, Pirro M. Endothelial and cardiac progenitor cells for cardiovascular repair: a controversial paradigm in cell therapy. Pharmacol Ther 2018;181:156–68.

35. Calabriso N, Stanca E, Rochira A, Damiano F, Giannotti L, Di Chiara Stanca B, Massaro M, Scoditti E, Demir C, Nitti P, Palermo A, Siculella L, Carlucco MA. Angiogenic properties of concentrated growth factors (CGFs): the role of soluble factors and cellular components. Pharmaceutics 2021;13:635.

36. Ito MT, da Silva Costa SM, Baptista LC, Carvalho-Siqueira GQ, Albuquerque DM, Rios VM, Ospari-Prieto S, Saez RC, Vieira KP, Cendes F, Ozel MC, Saad STO, Costa FF, Melo MB. Angiogenesis-related genes in endothelial progenitor cells may be involved in sickle cell disease. J Am Heart Assoc 2020;9:e014143.

37. Fan X, He L, Dai Q, He J, Chen X, Dai X, Zhang C, Sun D, Meng X, Sun S, Huang J, Chen J, Lin L, Chen L, Tan Y, Yan X. Interleukin-1β augments the angiogenesis of endothelial progenitor cells in an NF-κB/CXCR7-dependent manner. J Cell Mol Med 2020;24:5605–14.

38. Wakabayashi T, Naito H, Suehiro JI, Lin Y, Kawai J, Iba T, Kouno T, Ishikawa-Kato S, Furuno M, Takara K, Muramatsu F, Weizhen J, Kidoya H, Ishihara K, Hayashizaki Y, Nishida K, Yoder MC, Takakura N. CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. Cell Stem Cell 2018;22:384–97.e6.

39. Zhou Y, Li P, Goodwin AJ, Cook JA, Halushka PV, Chang E, Fan H. Exosomes from endothelial progenitor cells improve the outcome of a murine model of sepsis. Mol Ther 2018;26:1375–84.

40. Li Y, Wang J, Chen S, Wu P, Xu S, Wang C, Shi H, Bihl J. miR-137 boosts the neuroprotective effect of endothelial progenitor cell-derived exosomes in oxyhemoglobin-treated SH-SY5Y cells partially via COX2/PGE2 pathway. Stem Cell Res Ther 2020;11:330.

41. Nakaya M, Watari K, Tajima M, Nakaya T, Matsuda S, Ohara H, Nishihara H, Yamaguchi H, Hashimoto A, Nishida M, Nagasaka A, Horii Y, Ono H, Iribe G, Inoue R, Tsuda M, Inoue K, Tanaka A, Kuroda M, Agata S, Kuruse H. Cardiac myofibroblast engulfment of dead cells facilitates recovery after myocardial infarction. J Clin Invest 2017;127:383–401.

42. Eyrard SM, Leccce L, Michelle KC, Nomura-Kitabayashi A, Pandey G, Purushothaman KR, d’Escamard V, Li JR, Hadri L, Fujitani K, Moreno PR, Benard L, Rimmele P, Cohnain A, Mecham B, Randolph GJ, Nabel EG, Hajjar R, Fuster V, Boehm M, Kovacic JC. Endothelial to mesenchymal transition is
common in atherosclerotic lesions and is associated with plaque instability. Nat Commun 2016;7:11853.

44. Hou Z, Yan W, Li T, Wu W, Cui Y, Zhang X, Chen YP, Yin T, Qiu J, Wang G. Lactic acid-mediated endothelial to mesenchymal transition through TGF-b1 contributes to in-stent restenosis in poly-L-lactic acid stent. Int J Biol Macromol 2020;155:1589–98.

45. Shi J, Yang Y, Cheng A, Xu G, He F. Metabolism of vascular smooth muscle cells in vascular diseases. Am J Physiol Heart Circ Physiol 2020;319:H163–31.

46. Bongiorno T, Chojnowski JL, Lauderdale JD, Sulchek T. Cellular stiffness as a novel stemness marker in the corneal limbus. Biophys J 2016;111:1761–72.

47. Song L, Martinez L, Zigmond ZM, Hernandez DR, Lassance-Soares RM, Selman G, Vazquez-Padron RI. Vazquez-Padron RI. c-Kit modifies the inflammatory status of smooth muscle cells. PeerJ 2017;5:e3418.

48. Jeong Y, Yao Y, Yim EKF. Current understanding of intimal hyperplasia and effect of compliance in synthetic small diameter vascular grafts. Biomater Sci 2020;8:4383–95.

49. Tian B, Ding X, Song Y, Chen W, Liang J, Yang L, Fan Y, Li S, Zhou Y. Matrix stiffness regulates SMC functions via TGF-beta signaling pathway. Biomaterials 2019;221:119407.

50. Ibrahim A, Ciullo A, Li C, Akhmerov A, Peck K, Jones-Ag雄oo C, McIvor F, Lue F, Ebrahim AG. Engineered fibroblast extracellular vesicles attenuate pulmonary inflammation and fibrosis in bleomycin-induced lung injury. Front Cell Dev Biol 2021;9:733158.

51. Arishe OO, Priviero F, Wilczynski SA, Webb RC. Exosomes as intercellular messengers in hypertension. Int J Mol Sci 2021;22:11685.

52. Nathan C, Ding A. Nonresolving inflammation. Cell 2010;140:871–82.

53. Arishe OO, Priviero F, Wilczynski SA, Webb RC. Exosomes as intercellular messengers in hypertension. Int J Mol Sci 2021;22:11685.

54. Tauerz S, Starnes TW, Becker FB, Lam FY, Huttenlocher A. Redox and Src family kinase signaling control leukocyte wound attraction and neutrophil reverse migration. J Cell Biol 2014;207:589–98.

55. Xu J, Wang J, Qiu J, Liu H, Wang Y, Cui Y, Humphry W, Wang N, Durkan C, Chen Y, Lu Y, Ma Q, Wu W, Luo Y, Xiao L, Wang G. Nanoparticles retard immune cells recruitment in vivo by inhibiting chemokine expression. Biomaterials 2021;265:120392.

56. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 2016;44:450–62.

57. Guo L, Akahori H, Harari E, Smith SL, Polavarapu R, Karmali V, Otsuka F, Gannon RL, Braumann RE, Dickinson MH, Gupta A, Jenkins AL, Liptinski MJ, Kim J, Chhour P, de Vries PS, Jinnouchi H, Kutys R, Mori H, Kutyna MD, Torii S, Sakamoto A, Choi CU, Cheng Q, Grove ML, Sawan MA, Zhang Y, Cao Y, Kolidge FD, Cordon DE, Arking DE, Boerwinkle E, Morrison AC, Erdmann J, Sotodelnka N, Virmabi R, Finn AV. CD163+ macrophages promote angiogenesis and vascular permeability accompanied by inflammation in atherosclerosis. J Clin Invest 2018;128:1106–24.

58. Orecchiomi M, Gheosheh Y, Pramod AB, Ley K. Macrophage polarization: different gene signatures in M1(LPS+)- vs. Classically and M2(LPS–) vs. alternatively activated macrophages. Front Immunol 2019;10:1084.
Luo L, Zhang G, Liu B, Qin X, Zhang Y, Chen Y, Zhang H, Wu W, Wang G. Bioengineering CXCR4-overexpressing cell membrane functionalized ROS-responsive nanotherapeutics for targeting cerebral ischemia–reperfusion injury. Theranostics 2021;11:8043–56.

Wu G, Zhang J, Zhao Q, Zhuang W, Ding J, Zhang C, Gao H, Fang DW, Pu K, Xie HY. Molecularengineered macrophage-derived exosomes with inflammation tropism and intrinsic heme biosynthesis for atherosclerosis treatment. Angew Chem Int Ed Engl 2020;59:4068–74.

Xue Y, Zeng G, Cheng J, Hu J, Zhang M, Li Y. Engineered macrophage membrane-enveloped nanomedicine for ameliorating myocardial infarction in a mouse model. Bioeng Transl Med 2021;6:e10197.

Gao C, Huang Q, Liu C, Kwong CHT, Yue L, Wan JB, Lee SMY, Wang R. Treatment of atherosclerosis by macrophage-biomimetic nanoparticles via targeted pharmacotherapy and sequestration of proinflammatory cytokines. Nat Commun 2020;11:2622.

Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet-derived exosomes with inflammation tropism and intrinsic heme biosynthesis for atherosclerosis treatment. Angew Chem Int Ed Engl 2020;59:4068–74.

Su J, Sun H, Meng Q, Zhang P, Yin Q, Li Y. Enhanced blood susceptibility and Laser-Activated tumor-specific drug release of theranostic mesoporous silica nanoparticles by functionalizing with erythrocyte membranes. Theranostics 2017;7:523–37.

Gao W, Hu CM, Fang RH, Luk BT, Huang C, Zeng G, Cheng J, Hu J, Zhang M. Surface functionalization of gold nanoparticles with red blood cell membranes. Adv Mater 2013;25:3549–53.

Fan Z, Li PY, Deng J, Bady SC, Cheng H. Cell membrane coating for reducing nanoparticle-induced inflammatory responses to scaffold constructs. Nano Res 2018;11:5573–83.

Yu B, Kim HW, Wu S, Wang Y, Zhong Y, Tang D, Maruf A, Ye M, Wang L, Zhang P, Yin Q, Li Y. Enhanced affinity towards activated endothelium as versatile tools for theranostic drug delivery. Front Immunol 2019;10:1131–45.
105. Mause SF, Ritzel E, Liehn EA, Hristov M, Bidzhekov K, Müller-Newgen G, Soehnlein O, Weber C. Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. *Circulation* 2010;122:495–506.

106. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. *Int J Mol Sci* 2014;15:4142–57.

107. Chen L, Mou S, Hou J, Fang H, Zeng Y, Sun J, Wang Z. Simple application of adipo-derived stem cell-derived extracellular vesicles coating enhances cytocompatibility and osteoinductivity of titanium implant. *Regen Biomater* 2018;5:8038.

108. Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, Lim SK. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Adv Drug Deliv Rev* 2013;65:336–41.

109. Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X, Gao L, Xie J, Xu B. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia–reperfusion injury through miR-182-regulated macrophage polarization. *Cardiovasc Res* 2019;115:1205–16.

110. Li M, Xu Z, Zhang L, Cui M, Zhu M, Guo Y, Sun R, Han J, Song E, He Y, Su Y. Targeted noninvasive treatment of chordoid neo-vascularization by hybrid cell-membrane-coated biomimetic nanoparticles. *ACS Nano* 2021;15:9808–19.

111. Wang D, Dong H, Li M, Cao Y, Yang F, Zhang K, Dai W, Wang C, Zhang X. Erythrocyte-cancer hybrid membrane camouflaged hollow copper sulphide nanoparticles for prolonged circulation life and homotypic-targeting photothermal/chemotherapy of melanoma. *ACS Nano* 2018;12:5241–52.

112. Jiang Q, Liu Y, Guo R, Yao X, Sung S, Pang Z, Yang W. Erythrocyte-cancer hybrid membrane-camouflaged melanin nanoparticles for enhancing photothermal therapy efficacy in tumors. *Biomaterials* 2019;192:292–308.

113. Dehaini D, Wei X, Fang RH, Masson S, Angsamitkul P, Luk BT, Zhang Y, Ying M, Jiang X, Kroll AV, Gao W, Zhang L. Erythrocyte-platelet hybrid membrane coating for enhanced nanoparticle functionalization. *Adv Mater* 2017;29:1606209.

114. Bu LL, Rao L, Yu GT, Chen L, Deng WW, Liu JF, Wu H, Meng QF, Guo SS, Zhao XZ, Zhang WF, Chen GJ, Gu Z, Liu W, Sun ZJ. Cancer stem cell–platelet hybrid membrane-coated magnetic nanoparticles for enhanced photothermal therapy of head and neck squamous cell carcinoma. *Adv Funct Mater* 2019;29:1807733.

115. Rao L, Meng QF, Huang QQ, Wang ZX, Yu GT, Li A, Ma WJ, Zhang NG, Guo SS, Zhao XZ, Liu K, Yuan YF, Liu W. Platelet-leukocyte hybrid membrane-coated immunomagnetic beads for highly efficient and highly specific isolation of circulating tumor cells. *Adv Funct Mater* 2018;28:1803531.

116. Chen HY, Deng J, Wang Y, Wu CQ, Li X, Dai HW. Hybrid cell membrane-coated nanoparticles: a multifunctional biomimetic platform for cancer diagnosis and therapy. *Acta Biomater* 2020;112:1–13.

117. Hu S, Wang X, Li Z, Zhu D, Cores J, Wang Z, Li J, Mei X, Cheng X, Su T, Cheng K. Platelet membrane and stem cell exosome hybrid enhances cellular uptake and targeting to heart injury. *Nano Today* 2021;39:101210.

118. Zhong Y, Qin X, Wang Y, Qu K, Luo L, Zhang K, Liu B, Obaid E, Wu W, Wang G. “Plug and Play” functionalized erythrocyte nanoplatform for target atherosclerosis management. *ACS Appl Mater Interfaces* 2021;13:33862–73.

119. Tsoref O, Tyomin D, Armit U, Landa N, Cohen-Rosenboim O, Kain D, Golan M, Naftali-Shani N, David A, Leor J. E-selectin-targeted copolymer reduces atherosclerotic lesions, adverse cardiac remodeling, and dysfunction. *J Control Release* 2018;288:136–47.

120. Ui AIn Q, Chung H, ChungJY, ChoiJH, Kim YH. Amelioration of atherosclerotic inflammation and plaques via endothelial adrenocorticotargeted eNOS gene delivery using redox-sensitive polymer bearing L-arginine. *J Control Release* 2017;262:72–86.

121. Chung EJ, Minar LB, Nord K, Sugimoto MJ, Wonder E, Alenghat FJ, Fang Y, Tirrell M. Monocyte-targeting supramolecular micellar assemblies: a molecular diagnostic tool for atherosclerosis. *Adv Healthc Mater* 2015;4:367–76.

122. Luehmahn HP, Pressly ED, Detering L, Wang C, Pierce R, Woodard PK, Gropler RJ, Hawker CJ, Liu Y. PET/CT imaging of chemokine receptor CCR5 in vascular injury model using targeted nanoparticle. *J Nucl Med* 2014;55:629–34.

123. Uchida M, Kosuge H, Terashima M, Willits DA, Liepold LO, Young MJ, McConnell MV, Douglas T. Protein cage nanoparticles bearing the Lyp-1 peptide for enhanced imaging of macrophage-rich vascular lesions. *ACS Nano* 2011;5:2493–502.

124. Nie S, Zhang J, Martinez-Zagulian R, Sennoune S, Hossen MN, Lichtenstein AH, Cao J, Meyerrose GE, Paone R, Soontropa S, Fan Z, Wang S. Detection of atherosclerotic lesions and intimal macrophages using CD36-targeted nanovesicles. *J Control Release* 2015;220:61–70.

125. Kona S, Specht D, Rahimi M, Shah BP, Gilbertson TA, Nguyen KT. Targeted biodegradable nanoparticles for drug delivery to smooth muscle cells. *J Nanosci Nanotechnol* 2012;12:236–44.

126. Wang J, Hu L, Huang H, Yu Y, Wang J, Yu L, Li K, Li Y, Tian T, Chen F. CAR (CARKNNKD) peptide modified ReNcell-derived extracellular vesicles as a novel therapeutic agent for targeted pulmonary hypertension therapy. *Hypertension* 2020;76:1147–60.

127. Wang X, Chen Y, Zhao Z, Meng Q, Yu Y, Sun J, Yang Z, Chen Y, Li J, Ma T, Liu H, Li Z, Yang J, Shen Z. Engineered exosomes with ischemic myocardium-targeting peptide for targeted therapy in myocardial infarction. *J Am Heart Assoc* 2018;7:e008737.

128. Antes TJ, Middleton RC, Luther KM, Ijichi T, Peck KA, Liu WJ, Valle J, Echavez AK, Marbán E. Targeting extracellular vesicles to injured tissue using membrane cloaking and surface display. *J Nanobiotechnology* 2018;16:61.

129. Srinivasan R, Marchant RE, Gupta AS. In vitro and in vivo platelet targeting by cyclic RGD-modified liposomes. *J Biomed Mater Res A* 2010;93:1004–15.

130. Khaddaj Mallat R, Mathew John C, Kendrick DJ, Braun AP. The vascular endothelium: a regulator of arterial tone and interface for the immune system. *Crit Rev Clin Lab Sci* 2017;54:458–70.

131. Gimbrone MA, García-Cardeña G. Vascular endothelium, hemodynamics, and the pathology of atherosclerosis. *Cardiovasc Pathol* 2013;22:9–15.

132. Topper JN, Gimbrone MA Jr. Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. * Mol Med Today 1999*;5:40–6.

133. Vanhoutte PM. Endothelial dysfunction: the first step toward coronary arteriosclerosis. *Circ J* 2009;73:595–601.

134. Galkina E, Levy K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;27:2292–301.
myocardial infarction using cardiac homing peptide. Theranostics 2018;8:1869–78.

137. Wu H, Zheng J, Xu S, Fang Y, Wu Y, Zeng J, Shao A, Shi L, Lu J, Mei S, Wang X, Guo X, Wang Y, Zhao Z, Zhang J. Mer regulates microglial/macrophage M1/M2 polarization and alleviates neuroinflammation following traumatic brain injury. J Neuroinflammation 2021;18:2.

138. Li Q, Huang Z, Wang Q, Gao J, Chen J, Tan H, Li S, Wang Z, Weng X, Yang H, Pang Z, Song Y, Qian J, Ge J. Targeted immunomodulation therapy for cardiac repair by platelet membrane engineering extracellular vesicles via hitching peripheral monocytes. Biomaterials 2022;284:121529.

139. Stoger JL, Gijbels MJ, van der Velden S, Manca M, van der Loos CM, Biessen EA, Daemen MJ, Lugens E, de Winther MP. Distribution of macrophage polarization markers in human atherosclerosis. Atherosclerosis 2012;225:461–8.

140. Tabas I, Bornfeldt KE. Macrophage phenotype and function in different stages of atherosclerosis. Circ Res 2016;118:653–67.

141. Durham AL, Speer MY, Scatena M, Giachelli CM, Shanahan CM. Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. Cardiovasc Res 2018;114:590–600.

142. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013;200:373–83.

143. van Niel G, D’Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 2018;19:213–28.

144. Ma Y, Yang X, Chatterjee V, Meegan JE, Beard RS Jr, Yuan SY. Role of neutrophil extracellular traps and vesicles in regulating vascular endothelial permeability. Front Immunol 2019;10:1037.

145. Chen B, Li Q, Zhao B, Wang Y. Stem cell-derived extracellular vesicles as a novel potential therapeutic tool for tissue repair. Stem Cells Transl Med 2017;6:1753–8.

146. Todorova D, Simoncini S, Lacroix R, Sabatier F, Dignat-George F. Extracellular vesicles in angiogenesis. Circ Res 2017;120:1658–73.

147. Gardin C, Ferroni L, Erdoğan YK, Zanotti F, De Francesco F, Trentini M, Brunello G, Ercan B, Zavan B. Nanostructured modifications of titanium surfaces improve vascular regenerative properties of exosomes derived from mesenchymal stem cells: preliminary in vitro results. Nanomaterials (Basel) 2021;11:3452.

148. Vader F, Mol EA, Pasterkamp G, Schiellers RM. Extracellular vesicles for drug delivery. Adv Drug Deliv Rev 2016;106:148–56.

149. Xu R, Zhang F, Chai R, Zhou W, Hu M, Liu B, Chen X, Liu M, Xu Q, Liu N, Liu S. Exosomes derived from pro-inflammatory bone marrow-derived mesenchymal stem cells reduce inflammation and myocardial injury via mediating macrophage polarization. J Cell Mol Med 2019;23:7617–31.

150. Loyer X, Zlatanova I, Devue C, Yin M, Howangyin KY, Klahmmon P, Guerin CI, Kheloufi M, Vilar J, Zannis K, Fleischmann BK, Hwang DW, Park J, Lee H, Menasche P, Silvestre JS, Boulanger CM. Intra-cardiac release of extracellular vesicles shapes inflammation following myocardial infarction. Circ Res 2018;123:100–6.

151. Gray WD, French KM, Ghosh-Choudhary S, Maxwell JT, Brown ME, Platt MO, Searles CD, Davis ME. Identification of therapeutic covariant microRNA clusters in hypoxia-treated cardiac progenitor cell exosomes using systems biology. Circ Res 2015;116:255–63.

152. Wei Z, Qiao S, Zhao J, Liu Y, Li Q, Wei Z, Dai Q, Kang L, Xu B. miRNA-181a over-expression in mesenchymal stem cell-derived exosomes influenced inflammatory response after myocardial ischemia-reperfusion injury. Life Sci 2019;232:116632.

153. Born LJ, Harmon JW, Jay SM. Therapeutic potential of extracellular vesicle-associated long noncoding RNA. Bioeng Transl Med 2020;5:e10172.

154. Tang TT, Wang B, Wu M, Li ZL, Feng Y, Cao JY, Yin D, Liu H, Tang RN, Crowley SD, Liu L, Liu BC. Extracellular vesicle-encapsulated IL-10 as novel nanotherapeutics against ischemic AKI. Sci Adv 2020;6:eaa0748.

155. Dar GH, Mendes CC, Kuan WL, Speciale AA, Conceicao M, Gorgens A, Uliyakina I, Lobo MJ, Lim WF, El Andaloussi S, Mager I, Roberts TC, Barker RA, Goberdhan DCI, Wilson C, Wood MJ. GAPDH controls extracellular vesicle biogenesis and enhances the therapeutic potential of EV mediated siRNA delivery to the brain. Nat Commun 2021;12:6666.

156. Rayamajhi S, Nguyen TDT, Marasini R, Aryan S. Macrophage-derived exosome-mimetic hybrid vesicles for tumor targeted drug delivery. Acta Biomater 2019;94:482–94.

157. Qin X, Zhang K, Qiu J, Wang N, Qu K, Cui Y, Hu W, Wang Y, Wang G. Uptake of oxidative stress-mediated extracellular vesicles by vascular endothelial cells under low magnitude shear stress. Bioact Mater 2022;9:397–410.

158. Park JH, Jiang Y, Zhou JR, Gong H, Mohapatra A, Heo JY, Gao WW, Fang RH, Zhang LF. Genetically engineered cell membrane-coated nanoparticles for targeted delivery of dexamethasone to inflamed lungs. Sci Adv 2021;7:eabf7820.

159. Tang D, Wang Y, Wijaya A, Liu BY, Maruf A, Wang JX, Xu JX, Liao XL, Wu W, Wang GX. ROS-responsive biomimetic nanoparticles for potential application in targeted anti-atherosclerosis. Regen Biomater 2021;8:rbab033.