Apoptotic cells derived micro/nano-sized extracellular vesicles in tissue regeneration

Abstract: Extracellular vesicles (EVs), products released by cells in multiple biological activities, are currently widely accepted as functional particles and intercellular communicators. From the orthodox perspective, EVs derived from apoptotic cells (apoEVs) are responsible for cell debris clearance, while recent studies have demonstrated that apoEVs participate in tissue regeneration. However, the underlying mechanisms and particular functions in tissue regeneration promotion of apoEVs remain ambiguous. Some molecules, such as caspases, active during apoptosis also function in tissue regeneration triggered by apoptosis. ApoEVs are generated in the process of apoptosis, carrying cell contents to manifest biological effects, and possessing biomarkers to target phagocytes. The regenerative effect of apoEVs might be due to their abilities to facilitate cell proliferation and regulate inflammation. Such regenerative effect has been observed in various tissues, including skin, bone, cardiovascular system, and kidney. Engineered apoEVs are produced to amplify the biological benefits of apoEVs, rendering them optional for drug delivery. Meanwhile, challenges exist in thorough mechanistic exploration and standardization of production. In this review, we discussed the link between apoptosis and regeneration, current comprehension of the origination and investigation strategies of apoEVs, and mechanisms in tissue regeneration by apoEVs and their applications. Challenges and prospects are also discussed here.

Keywords: extracellular vesicles, apoptosis, tissue regeneration, biomaterials, engineered nanoparticles

Abbreviations

AiP apoptosis-induced compensatory proliferation
ApoBDs apoptotic bodies
ApoEVs extracellular vesicles derived from apoptotic cells
ApoExos apoptotic exosomes
ApoMVs apoptotic microvesicles
BMM bone marrow macrophages
EVs extracellular vesicles
FACS fluorescence activated cell sorting
HUVEC human umbilical vein endothelial cells
MSCs mesenchymal stem cells
MSNs mesoporous silica nanoparticles
PtdSer phospholipid phosphatidylserine
PTC proximal tubular cell
ROS reactive oxygen species
UV ultraviolet

1 Introduction

Extracellular vesicles (EVs) are micro/nano-sized vesicles containing small particles with lipid membranes secreted...
by cells in multiple biological activities. EVs serve as information communicators of cells [1,2], which were formerly considered as cell debris. According to biogenesis and size, EVs can be roughly classified as exosomes (endosome origin, 30–100 nm in diameter), microvesicles (plasma origin, 50–1,000 nm), and apoptotic bodies (apoptotic cell origin, 1–5 μm) [3,4]. EVs of various sizes from apoptotic cells have been studied over the last several decades, and they are not just confined to apoptotic bodies. As a result, apoptotic vesicles are used to refer to all types of EVs derived from apoptotic cells (apoEVs) [5–7].

ApoEVs, as the products and information disseminators, seem to inherit the therapeutic role of apoptosis in tissue regeneration, changing death to another beginning of life [8,9]. Different from necrosis, apoptosis is unlikely to cause a traumatic defect or aberrant tissue shrinkage. As a result, apoptosis is well-known to play a role in tissue homeostasis and life cycle regulation, although there are still many unsolved mysteries in the field [10]. In recent years, tissue renewal, such as epithelium, skin, and liver renewal, has been widely described as one of the major biological effects mediated by apoptosis [11–13]. ApoEVs are products in apoptotic process. Traditionally, the uptake of apoEVs by phagocytes was thought to be the end of apoptosis, which biochemically signals the death of cells, but new studies suggest that their fates include transfer and reuse [14–17]. Apoptosis-like regeneration properties have also been demonstrated in the application of apoEVs, which come in a variety of sizes and are produced through different apoptotic pathways. Recent studies indicate that apoEVs might promote regeneration in tissue maintenance, including skin, bone, cardiovascular system, and kidney, by functioning bioactive effects through recruitment of targeted cells and delivery of biomolecules [18–22]. To better utilize and amplify the therapeutic properties of apoEVs, engineered methods implicating targeting, releasing, and retaining have been investigated [23,24]. Figure 1 demonstrates the production, isolation, functions, and applications of apoEVs. Culture medium of apoptotic cells was collected and centrifuged to obtain apoEVs. By recruiting targeting cells and delivering biomolecules, apoEVs promote cell proliferation and regulate inflammation, thus being applied in tissue regeneration. ApoEVs are modified to function in broader fields as well. Recent studies have discussed the functions and applications of apoEVs in immune regulation, inflammation mediation, mesenchymal stem cell (MSC) transplantation, and tumor progression [3,25–28]. Nonetheless, few documents have comprehensively discussed the regenerative effect of apoEVs and analyzed the underlying mechanisms. In this review, the mechanisms of apoEVs in tissue regeneration are addressed, along with their applications in both medicine and engineering. We hope this work will provide an indicative view of the mechanisms in tissue regeneration by apoEVs, and inspiration for novel application of apoEVs in the future.

2 Apoptosis and regeneration

2.1 Apoptosis with caspases

Since the term “apoptosis” was proposed for the first time by John Foxton Ross Kerr and his colleagues at the University of Queensland [29], it has been proven to participate in various physiological processes, including development, aging, immune homeostasis, and tissue integrity maintenance [3,16,30]. Apoptosis, the most prominent pattern of programmed cell death, is characterized by several morphological changes, involving cell shrinkage, cytoplasmic and nuclear condensation, membrane blebbing, and the subsequent formation of apoptotic bodies.

Apoptosis, a conserved physiological process, can typically be triggered by intrinsic pathway and extrinsic pathway. A general summary is discussed here to clarify the processes of apoptosis. Caspases are fundamental participants in both pathways of apoptosis. The intrinsic pathway of apoptosis is closely associated with mitochondrial regulation, in which stimuli (for example, DNA damage) generate biochemical changes inside cells. Stimuli elicit the dissipation of mitochondrial transmembrane potential, thus forming mitochondrial permeability transition pore on the outer membrane and leaking proapoptotic mitochondrial contents. Cytochrome c is then released into the cytoplasm to induce oligomerization of apoptotic peptide activating factor 1, which recruits procaspase-9 and forms apoptosome, the activator of caspase-9. Subsequently, activated caspase-9 in turn cleaves response caspases, activating caspase cascade reactions [16,31,32]. The extrinsic pathway of apoptosis initiates activation of death receptors. Upon contact with extracellular ligands, mainly TNF and Fas, the death receptors on the cell membrane transfer death signals towards the cytoplasm. The binding of ligands and receptors initiates recruitment of Fas-associated death domain protein, causing autocleavage of procaspase-8. Activated caspase-8 then cleaves procaspase-3 to yield activated caspase-3 responsible for executing downstream degradation process [16,30,32].
2.2 Regeneration with apoptosis

The mechanism of apoptosis is much more sophisticated than the brief sketch above, concerning a series of interactions and cascade signaling pathways, in which caspases have a well-defined role. However, increasing evidence implies the function of caspases in tissue repair and regeneration [33]. Dead cells are replaced by compensatory proliferated cells to maintain tissue homeostasis. Apoptotic cells deliver mitogenic signals to trigger the compensatory proliferation of neighboring cells, which is so-called “apoptosis-induced compensatory proliferation (AiP)” [11,34].

Caspases, the initiator and effector of apoptosis, are the major activators of AiP [11]. In Drosophila, cell death was blocked by effector caspase inhibitor p53 even with high expression of initiator caspase Dronc, rendering Dronc functioning apart from apoptosis [35,36]. Actually, Dronc activated AiP via JNK signaling pathway to express Wingless and Decapentaplegic mitogens, triggering compensatory proliferation of surrounding cells [34,37]. In Dronc-induced AiP, extracellular reactive oxygen species (eROS) produced by NADPH oxidase Duox activated macrophage-like hemocytes, which in turn amplified the JNK signal by TNF ortholog Eiger, thus promoting epithelial cell growth [11,12]. Effector caspases DrICE and Dcp-1 induced AiP in eye tissue via Hedgehog signaling [36,38].

In some other species, such as Xenopus tadpole and Hydra, ROS are pivotal compartments for AiP. Profuse ROS at the wound activated Wnt/β-catenin signaling and downstream fgf20, initiating active cell proliferation in tail regeneration of Xenopus tadpole [39]. In Hydra, ROS produced immediately after injury elicited MAPK signaling [11] and Wnt3 [40] in head regeneration.

In mammals, regeneration due to AiP is also linked to caspases. In mice, the lack of caspase-3 or caspase-7 leads to deficiency in skin and liver regeneration [13]. Cleaved caspase-3 and caspase-7 initiate calcium-independent phospholipase A2 to upregulate the expression of arachidonic acid, which further mediates the production of prostaglandin E2, facilitating tissue repair and stem cell proliferation [13]. The activation of caspase-3 enlarged YAP-dependent organs, while inhibition of caspase-3 attenuated cell proliferation and diminished sebaceous gland [41]. Some mediate evidence also proves the function of caspases in regeneration. The self-renewal property of embryonic stem cells was remarkably inhibited when lacking caspase-3 [42]. Deficient caspase-3 in mice resulted in impaired differentiation of bone marrow stem cells and decreased bone density by activation of TGF-β/Smad2 signaling pathway [43,44], which was ameliorated by secretion from apoptotic MSCs [44]. In conclusion, participants in apoptosis, such as caspases and ROS, function in tissue renewal, coupling cell death and regeneration.

Figure 1: The production, isolation, functions, and applications of apoEVs. Culture medium of apoptotic cells was collected and centrifuged to obtain apoEVs. By recruiting targeting cells and delivering biomolecules, apoEVs promote cell proliferation and regulate inflammation, thus being applied in tissue regeneration. ApoEVs are modified to function in broader fields as well.
2.3 ApoEVs

ApoEVs, the products of the programmed cell clearance process, are intercellular signal transporters harboring biomolecules from dying cells, which were previously recognized as cell debris [8,25]. Traditionally, apoEVs are indicated to be apoptotic bodies with a diameter of 1–5 µm [8,29,32]. Some smaller extracellular vesicles are also generated in apoptosis, described as apoptotic microvesicles (apoMVs, 50–1,000 nm) [4,7,28,44] and apoptotic exosomes (apoExos) (<150 nm) [6,45], because their size resembles microvesicles and exosomes released by viable cells [46]. Since the conventional identification of subtypes of apoEVs is currently ambiguous [25], apoEVs could be a general description of micro–nanoscale vesicles [47]. Here we demonstrated several representative morphologies of apoEVs (Figure 2).

2.4 Biogenesis and characteristics

Nowadays, biogenesis of apoEVs is described as three sequential well-coordinated steps with corresponding morphological changes: plasma membrane blebbing, thin membrane protrusions formation, and fragmentation [8,25]. In membrane protrusions formation, different protrusions might be generated, including microtubule spike, apoptopodia, and beaded apoptopodia. However, for some specific cells, not every step is necessary for the biogenesis of apoEVs (Figure 3).

Apoptotic membrane blebbing, the earliest morphological change in apoptosis, is driven by cytoskeleton collapse and increased hydrostatic pressure [32]. Caspases and various protein kinases regulate this mechanism, preventing apoptosis when they are inhibited [31]. Rho-associated protein kinase 1, a target of active caspase-3, induces phosphorylation of myosin light chain (MLC), causing actomyosin contraction [32]. MLC kinase facilitates the assignment of degraded chromatin into blebs [48]. Serine/threonine LIM domain kinase 1 inhibits the actin-binding protein coflin to promote actin polymerization [49]. This membrane blebbing is known as fundamental for membrane protrusions, but some cells undergo different membrane deformation in this process, indicating diverse mechanisms in addition to blebbing solely in apoEVs formation (Figure 3). Apoptotic A431 epithelial cells formed a rigid membrane protrusion, the microtubule spike, in the process of apoptosis without blebbing [50]. Human Jurkat T cells generated apoptopodia following blebbing, which is regulated by the caspase-activated

Figure 2: Several representative images of apoEVs. (a) Scanning electron microscope image of MSCs-derived apoEVs. Scale bar, 1 µm [18]. (b) Transmission electron microscope image of MSCs-derived apoEVs. Scale bar, 125 nm [68]. (c) Live differential inference contrast microscopy image showing generation of apoEVs from THP-1 cells undergo UV-induced apoptosis. Scale bar, 5 or 10 µm as indicated [5].
Recent, an novel “beads-on-a-string” type of membrane protrusion in apoptosis was described in apoptotic human THP-1 cells and monocytes. This beaded apoptopodia forms abundant apoEVs (approximately 10–20) with diameters of 1–4 µm due to the formation of protrusions on the “string” with a length 8 times that of the dying cell, rendering it much more efficient than the previous 2 patterns of membrane protrusion[52]. After blebs and/or membrane protrusions formation, fragmentation is responsible for the drop of single apoEV (Figure 3). While the specific mechanism or molecules mediating this process are currently unclear now, shear force in circulation or intercellular physical force of cell disassembly might be involved[8].

By the end of apoptosis, “find-me” and “eat-me” signals are indispensable for attraction and clearance of phagocytes[53], avoiding cell contents leakage and secondary necrosis[32]. The “find-me” signal is chemo-attractive gradient generated by released soluble molecules in apoptosis, such as ATP, UTP[54], and CX3CL1/fractalkine[55], which was also found to be associated with apoEVs[25,56]. Some receptor proteins in the membrane of phagocytes, the G protein-coupled receptor G2A for example, induce the recognition of “find-me” signals, even though the precise pathway of this process and quantified attractive gradient remain to be elucidated[57]. The most universally studied “eat-me” signal is the exposure of phospholipid phosphatidylserine (PtdSer)[55], anchoring on the surface of apoEVs. In the membrane of healthy cells, PtdSer is strictly confined to the inner side of the bilayer. While undergoing apoptosis, PtdSer flip from the inner side of the lipid bilayer of plasma membrane to the outer layer during blebbing, which is induced by caspase-activated scramblases, a set of phospholipid translocases[57]. The appearance of PtdSer on the outer layer in both apoptotic cells and apoEVs transmits the dying signal, inducing recognition and clearance of phagocytes. In addition, ICAM-3, a confirmed mediator of apoptotic cell clearance, was also present on the surface of apoEVs to attract macrophages[58]. Notably, apoEVs seem
to recruit phagocytes selectively, which might be due to content diversity caused by different parental cells and specific physiological and pathological processes. Adipocyte-derived apoEVs recruited macrophages rather than neutrophils [59]. However, neutrophils infiltration was observed in mice, in which larger apoEVs (>1µm in diameter) attracted neutrophils, whereas smaller ones (<1 µm) could not. Inflammatory chemokine IL-1 appeared in larger apoEVs but not in smaller ones to induce sterile neutrophil inflammation, which might originate from the nuclear IL-1α precursor that translocated into the nucleus during apoptosis [60]. The equilibrium between apoEVs and phagocytes is indispensable for homeostasis. Secondary necrosis might occur when massive apoptosis and subsequent excessive apoEVs containing inflammatory factors overwhelm the clearance ability of phagocytes or when the function or quantity of phagocytes is impaired in diseases.

In most studies, apoEVs were described as bioactive carriers transporting functional molecules [3,16,31], including DNA debris [61], RNA [21,62–64], and proteins [65,66], yet cargo differences among each group of apoEVs exist widely. The proteomes of healthy and cirrhotic human biliary epithelial cells showed a huge divergence in the shotgun proteomics of apoEVs [65]. Furthermore, exosome-like apoEVs carried more active 20 S proteasome core than that in apoptotic bodies (apoBDs), which is responsible for their immunogenic activity [45]. More interestingly, DNA and RNA could not be packed into apoEVs of HL-60 cells simultaneously. Over 90% of apoEVs containing RNA were free of DNA, and vice versa [62]. Additionally, nuclear material was absent in beaded apoptopodia [52], suggesting that some bioactive molecules may enter apoEVs with tropism. Different groups of apoEVs possess diverse properties and functions due to their distinct biogenesis processes, and unequal contents that result from different parental cells and their previous physiological and pathological changes.

2.5 Production and identification

Technically, it is necessary for researchers to study apoEVs in a relatively uniform condition to acquire convincing information, whereas cells in a general culture condition are less likely to undergo apoptosis under control. Thus, some stimuli of apoptosis in specific physiological or pathological conditions have been applied in studies to produce apoEVs. Here we reviewed production and isolation methods in recent years studying apoEVs, along with their documented times, donor cells, and experimental models (Table 1). The most universally used stimulus of apoEVs is staurosporine [18,19,23,64,67,68], a potent inhibitor of protein kinase C, which induces apoptosis by activating caspase-3 and suppressing AKT/MAPK pathway [69,70]. Serum starvation might affect cell cycle and promote apoptosis [71]. Exposure to gamma rays or ultraviolet radiation injured the stability of nucleic acids [67,72,73], which appeared when applying some chemotherapeutic drugs, such as cisplatin [67]. For some specific cells, the corresponding stimulus was applied. Alendronate was used to produce apoEVs from osteoclasts, due to endoplasmic reticulum stress in osteoclast precursors caused by alendronate [74].

For isolation of apoEVs, apoMVs, a subtype of apoEVs resembling microvesicles in size [4], were obtained in a centrifuge at 16,000–50,000g. ApoBDs and apoExos were isolated in a centrifuge at 1,000–5,000g and 100,000–200,000g, respectively. The size and morphology were observed and determined by dynamic light scattering analysis, scanning electron microscope, transmission electron microscope, or confocal microscope. Fluorescence-activated cell sorting (FACS) was used to quantify and purify apoEVs. Several molecules were detected to identify apoEVs. The most widely accepted biomarker is Annexin V, a calcium-binding protein of the annexin superfamily, which can interact with the “eat-me” signal PtdSer. Thrombospondin and complement protein C3b, the products of membrane changes during apoptosis, are also well-accepted biomarkers. Cleaved caspase-3 and C1q were also applied in some cases. Nuclear granularity and hypochromicity were detected by propidium iodide, but their quantities were not equal and were even absent in some apoEVs [5,15,18–21,23,44,63,64,67,68,72,73,75].

However, it is noteworthy that the current classification is based on size, while ignoring their biogenesis in cells and the contents of each group. Besides, apoEVs derived from different cells might differ in size. And apoEVs would collapse over time in vitro [5]. Actually, current studies are less likely to identify and analyze the subtype properties of apoEVs utilized in experiments, rendering disturbances from different groups being inevitable. It is pivotal to further analyze the contents and properties and uncover the key proportion of each group of apoEVs, which contributes to standardizing protocols for isolation and identification, thus obtaining rigorous scientific results with diminished confounding.

3 Functions of apoEVs in tissue regeneration

ApoEVs regulate cell function by transferring various abundant cargo, which involves many bioactive factors
Table 1: Production and isolation strategies of apoEVs in recent years

| Study                  | Origin of apoEVs                        | Inducer of apoptosis     | Models                  | Isolation strategy                                      |
|------------------------|-----------------------------------------|--------------------------|-------------------------|---------------------------------------------------------|
| Hristov et al. [118]   | HUVEC                                   | Serum starvation         | In vitro: epithelial progenitor cell | 16,000g*                                                 |
| Zernecke et al. [21]   | HUVEC; human atherosclerotic plaque material and mouse whole blood | Serum starvation         | In vitro: HUVEC         | 16,000g for supernatant; FACS for tissue and blood     |
| Zhu et al. [63]        | BMM                                     | Lipopolysaccharide       | In vitro: A549 epithelial cell | 2,000g                                                  |
| Shen et al. [72]       | T cell and neutrophil                   | UV exposure              | In vitro: Th cell       | 100,000g                                                |
| Liu et al. [44]        | MSC                                     | Staurosporine            | In vitro: HUVEC         | 1 and 5 µm filters followed by 2,000g                  |
| Brock et al. [75]      | Epithelial stem cell                    | Metronidazole            | In vivo: zebrafish      | —                                                       |
| Hardy et al. [64]      | HUVEC                                   | Serum starvation         | —                       | 50,000g for apoBDs and 200,000g for apoExos             |
| Chen et al. [73]       | Primary murine thymocytes and Jurkat cell | Gamma ray or UV exposure and serum starvation | In vitro: macrophage | 180,000g                                                |
| García-Pastor et al. [67] | HK-2 cell                          | Cisplatin and UV exposure | In vitro: HK-2 cell    | 5,000g                                                  |
| Ma et al. [20]         | BMM and osteoclast                      | Alendronate              | In vitro: MC3T3-E1 cell | 1 and 5 µm filters followed by 3,500g                  |
| Tyukavin et al. [90]   | Cardiomyocyte and fibroblast            | Serum starvation         | In vivo: rat            | 16,500g                                                 |
| Liu et al. [15]        | MSC                                     | Staurosporine            | In vitro: HUVEC         | 16,000g                                                 |
| Ma et al. [19]         | Osteoclast                              | Staurosporine            | In vivo: endothelial cell | 3,000g                                                  |
| Dou et al. [23]        | T cell and HUVEC                       | Staurosporine            | In vivo: macrophage     | 1,000g                                                   |
| Liu et al. [18]        | MSC                                     | Staurosporine            | In vivo: macrophage     | 16,000g                                                 |
| Zheng et al. [68]      | MSC                                     | Staurosporine            | In vivo: macrophage     | 16,000g                                                 |

*Centrifuge speed; HUVEC: human umbilical vein endothelial cells; FACS: fluorescence activated cell sorting; BMM: bone marrow macrophages; UV: ultraviolet; MSC: mesenchymal stem cells.
participating in tissue repair and regeneration. ApoEVs derived from different parental cells might differ in content and affect cell activities via diverse pathways. In tissue regeneration, beneficial effects of apoEVs have been studied and discussed. Figure 4 demonstrates the main mechanisms of apoEVs in tissue regeneration, including promoting the proliferation of neighboring cells by compensatory proliferation signals and remote cells by transferring therapeutic molecules, and suppressing inflammation by regulating macrophage polarization (Figure 4).

**3.1 Promoting cell proliferation**

Tissue integrity is maintained in the homeostasis of dynamic cell death and compensatory proliferation. Apoptosis has been implied to activate compensatory proliferation signaling, hence dying cells mediate compensatory proliferation in adjacent cells [11,13,30,34,76,77], which is also called AiP [11,34]. ApoEVs, the product of dying cells, seem to inherit such a compensatory proliferation function. ApoEVs produced by different apoptotic stimuli were reported to trigger neighboring cell proliferation in both transformed and primary cells upon contact via compensatory proliferation signaling [78], which is related to CrkI, a type of protein responsible for cell proliferation and cytokinesis [79,80] (Figure 4). Epithelial stem cell-released apoEVs also triggered cell proliferation of standby healthy stem cells in a compensatory proliferation way, maintaining regeneration of the epithelium [75]. In addition to the near approach, apoEVs were also implicated in promoting cell proliferation in remote sites. ApoEVs from macrophages delivering miR-221/222 induced lung epithelial cell growth by suppressing the expression of cyclin-dependent kinase inhibitor 1B, a tumor suppressor controlling cell cycle at G1 [63] (Figure 4). Circulating apoEVs from MSCs transferring ubiquitin ligase RNF146 and miR-328-3p recovered the self-renewal and differentiation properties of distant impaired MSCs [44] (Figure 4). Intriguingly but predictably, Wnt/β-catenin signaling pathway participated closely in cell growth and renewal promoted by apoEVs [44,75,78]. Focusing on

**Figure 4:** The main mechanisms of apoEVs in tissue regeneration, including promoting the proliferation of neighboring cells by compensatory proliferation signals and remote cells by transferring therapeutic molecules, and suppressing inflammation by regulating macrophage polarization.
miRNAs and proteins modulating Wnt signaling pathway in apoEVs could be a possible way to elucidate the mechanisms by which apoEVs promote tissue regeneration.

### 3.2 Regulating inflammation

Inappropriate inflammation impedes repair and regeneration progress in multiple diseases [81–84]. ApoEVs are capable of attenuating inflammation by modulating cell activities of inflammatory cells, thereby smoothing the tissue regeneration process. Suppression of inflammatory cytokines, including MCP-1 and IL-6, caused by upregulated TSG-6 was observed after treatment with apoptotic MSCs [85] (Figure 4). TSG-6 has been reported to reduce proinflammatory signals from macrophages [86] and inhibit the activation of macrophages [87]. Furthermore, apoEVs have been implicated in modulating macrophage polarization both in vivo and in vitro. In mouse models, apoEVs from MSCs promoted polarization of anti-inflammatory M2 macrophages, whereby they increased the anti-inflammatory cytokines IL-10 and TGF-β and enhanced the migration and proliferation of fibroblasts [18] (Figure 4). Biomimic apoEVs were taken up more effectively by activated M1 macrophages and led to more intensive M2 macrophage polarization, ameliorating inflammation by upregulating anti-inflammatory cytokines [88]. In addition, apoEVs derived from activated T cells were likely to possess trophic property to inflammatory site similar to that of their parental cells, meanwhile promoting M2 macrophage polarization [23]. In short, apoEVs help to recover a healthy microenvironment of repair and regeneration by ameliorating excessive local inflammation.

Besides above all, many other intricate mechanisms contribute to the regeneration function of apoEVs. ApoEVs from MSCs promoted the proliferation of endothelial cells [15] and endothelial progenitor cells [89], thus inducing angiogenesis. ApoEVs derived from different parental cells manifested specific functions in regeneration resembling their parental cells, which was observed in preosteoclasts and mature osteoclasts [19,20], cardiomyocytes [90,91], and proximal tubular cells [67]. From this perspective, stem cells might be a promising origin of apoEVs due to their intrinsic regenerative properties. However, it is difficult to draw a definite conclusion from these scattered studies, since there is little convincing result indicating the direct correlation between the regenerative property of apoEVs and specific mechanisms.

### 4 Therapeutic effects of apoEVs in regeneration

In recent years, researchers have probed and utilized the regeneration ability of apoEVs in numerous tissue regeneration processes. Here we summarize and discuss the
promotion of skin, bone, heart, and kidney repair and regeneration by apoEVs (Figure 5).

4.1 ApoEVs in skin regeneration

ApoEVs produced by MSCs facilitated wound healing as MSCs [18,85,92]. ApoEVs induced M2 macrophage polarization, the anti-inflammatory subtype of macrophages, and sequentially promoted the viability of fibroblasts, accelerating cutaneous wound repair [18] (Figure 5). Furthermore, apoEV mimics carry miR-21/curcumin boosted M2 polarization [23], indicating that the membrane of apoEVs and these therapeutic factors cooperated in skin regeneration. Taking advantage of the macrophage targeting property, apoEVs delivering antibiotic vancomycin relieved intracellular methicillin-resistant *Staphylococcus aureus* infection of macrophages [24]. Remaining dormant in macrophages, intracellular *Staphylococcus aureus* escape from immune system and antibiotics, causing persistent cutaneous infection, which results in impaired wound healing and skin regeneration [93]. Moreover, after the apoptotic induction of TNF-α, apoEVs enveloping more IL-1RA were released by MSCs, promoting gingival and skin regeneration through Fas/Fap-1/Cav-1 axis [92]. Reduced hypertrophic scar formation in the process of skin regeneration was also observed after treatment with TSG-6 secreted by apoptotic human MSCs [85]. TSG-6, a protective factor in the inflammatory response, has been reported to be one of the dominant mediators in the regenerative ability of MSCs [94].

4.2 ApoEVs in bone regeneration

ApoEVs produced by osteoclasts also manifested similar biological effects as parental cells [19,20]. Preosteoclast-derived apoEVs promoted angiogenesis, while mature osteoclast-apoEVs promoted osteogenesis, similar to perspective parental cells both in bioinformatics analysis and in vivo [19]. Mechanistically, mature osteoclast-derived apoEVs induced the differentiation of osteoblasts that mediate osteogenesis by activating receptor activator of NF-κB ligand reverse signaling [20,95], coupling bone resorption and formation (Figure 5). Notably, MSCs-released apoEVs rescued the osteopenia phenotype in both apoptosis-deficient mice and ovariectomized mice by restoring stem cell properties of impaired MSCs (Figure 5). The uptake in circulation of ubiquitin ligase RNF146 and miR-328-3p carried by apoEVs activated Wnt/β-catenin pathway [44], a conservative signaling pathway crucial in development and tissue regeneration [96,97]. These results demonstrate the indispensable role of apoEVs in regulating bone remodeling and maintaining bone homeostasis.

4.3 ApoEVs in cardiovascular system

ApoEVs derived from different donor cells provide varied biological benefits for cardiovascular system protection and regeneration. Endothelial apoEVs deliver miR-126 ameliorated atherosclerosis and plaque in vivo by reducing macrophage infiltration and apoptotic cells. Endothelial apoEVs upregulated the expression of chemokine CXCL12 and mobilized Sca-1+ progenitor cells, thus inhibiting CXCR4-dependent atheroproggression [21]. Similarly, an apoEV bio-mimic liposome enhanced the stability of atherosclerotic plaques, which means there is a lower possibility of plaque rupture and subsequent thrombosis by improving macrophage-induced inflammation, to protect cardiovascular system [88] (Figure 5). In another aspect, MSC-derived apoEVs enhanced angiogenesis and cardiac function recovery in myocardial infarction mice, which was due to recipient endothelial cells activating autophagy [15]. Moreover, apoEVs derived from cardiomyocyte regulated the differentiation of cardiac stem cells to cardiomyocyte precursors and improved the contractility of myocardium [90,91], while apoEVs released by fibroblast produced no such effect [90] (Figure 5).

4.4 ApoEVs in renal regeneration

In renal regeneration, a novel type of apoEVs containing CrkI has been described, inducing mitosis and proliferation of nearly 100% analyzed recipient cells including renal parietal epithelial cells [78]. Podocyte-derived apoEVs containing CrkI might promote regeneration of injured tubular epithelial cells [22] (Figure 5). Surprisingly, apoEVs released by the first apoptosis in proximal tubular cells (PTCs) caused by cisplatin mediated the secondary apoptosis of recipient PTCs, while apoEVs produced by this secondary apoptosis stimulated tubular cell proliferation [67]. These controversial effects of apoEVs derived from different apoptosis processes might be explained by propagation of toxic effects of cisplatin, or apoptotic stimulus of Fas ligand that probably expressed on apoEVs [98]. Nevertheless, specific comparison experiments concerning apoEVs induced by cisplatin and other stimuli, and the interaction and
### Engineered apoEVs

EVs have been reported to possess tropism targeting a specific tissue or cell [99–103], known as the “homing effect” [101–103]. Moreover, EVs show natural advantages as drug delivery systems over synthetic nanoparticles, with the abilities to cross biological barriers efficiently and interact with the plasma membrane via ligand/receptor responses, while possessing low immunogenicity and toxicity [100,104–107]. These intrinsic properties of apoEVs qualify them as optimal bioactive materials to be modified for tissue regeneration, causing more precise tissue targeting, enhanced inflammation modulation, and longer drug retention. Thus, apart from the direct utilization of natural apoEVs, modified apoEVs or biomimetic apoEVs are also a concern.

The unique identifying signal of apoEVs for phagocytes has piqued the interest of researchers in harnessing their targeting ability. ApoEV bio-mimic liposomes were constructed by connecting PtdSer onto the surface of liposomes [88], inspired by the “eat-me” signal from exposed PtdSer of apoEVs. The PtdSer-modified liposomes effectively escaped from clearance by reticuloendothelial system owing to PEGylation [88,108]. Compared with liposomes, the modified liposomes that targeted M1 macrophages were engulfed more effectively as expected. As a sign of suppressed inflammation, inhibited M1 polarization and motivated M2 polarization were observed from increased anti-inflammatory cytokines such as IL-4 and IL-10 and decreased proinflammatory cytokines such as IL-1β, IL-6, and TNF-α [88] (Table 2). In addition, apoEVs derived from some kinds of cells might inherit the particular targeting property of their parental cells [109], such as leukocytes. Taking this into consideration, apoEVs released by T cell were manipulated to target inflammatory sites and then macrophages [23]. In this research, natural membranes of T cell derived apoEVs without cargos were obtained, and then encapsulated with mesoporous silica nanoparticles (MSNs) that were preloaded with anti-inflammatory molecules. These chimeric apoEVs showed CD44 and Mac-1 antigen targeting ability. ApoEVs carrying vancomycin delivered drugs into cells [112,113], thus displaying inflammation targeting capacity both in vitro and in vivo.

#### 5 Engineered apoEVs

| Methods of modification | Forms of modification | Functional parts | Purposes of modification | Results of modification |
|-------------------------|----------------------|-----------------|-------------------------|------------------------|
| Connecting PtdSer onto the surface | Liposomes with “eat-me” signal of apoEVs | PtdSer on the surface | Targeting property for macrophages | Lipid membrane of apoEVs; antibacterial effect inside macrophages |
| Co-bathing in sonication of | Chimeric T-cell apoEVs with MSNs loading | CD44, and Mac-1 | Targeting property for inflammatory sites | Anti-inflammatory effect inside macrophages; showed CD44 and Mac-1 antigen targeting ability |
| Frezing and thawing apoEVs with | Lipid membrane of apoEVs; vancomycin | Anti-inflammatory molecules | delivering drugs into cells | Proved possibility of loading miRNA in apoEVs in improved skin regeneration |
| Transient transfection of miRNA into donor cells | Reconstructed apoEVs carrying miRNAs | Therapeutic miRNAs | Loading and delivery of miRNA | Sustained release of apoEVs in inflammatory sites |
| Embedding apoEVs into PF127 | Reconstructed apoEVs carrying vancomycin | Anti-inflammatory molecules | delivering drugs into cells | Improved anti-inflammatory cytokines such as IL-4 and IL-10 and decreased proinflammatory cytokines such as IL-1β, IL-6, and TNF-α |

PtdSer: phosphatidylserine; MSN: mesoporous silica nanoparticles; miRNA: micro-RNA; siRNA: silencing RNA.

Table 2: Modification of engineered apoEVs in recent years
in colitis models (Table 2). Promoted macrophage-specific phagocytosis and M2 polarization were observed as well [23]. Similarly, cancer cell derived apoEVs were reconstructed for targeting the same type of cancer cells, as well as targeting macrophages [24].

Combined with their targeting property dictated by their membrane markers and cargo content [105], the natural lipid membrane potentialized apoEVs as drug delivery systems. Co-bathing in sonication is one of the common strategies to load therapeutic molecules into apoEVs [23] (Table 2). To boost the efficiency and stability of drug loading, therapeutic molecules could be pre-loaded into MSNs, an outstanding nanoparticle as a drug carrier [112,113], by electrostatic interactions [23] or undergoing a freeze–thaw process bathing with apoEVs [24]. By a mechanically extruding technique, apoEVs were processed into a relatively uniform size of 80–150 nm and obtained an enhanced encapsulation efficiency of vancomycin [24] (Table 2). Otherwise, genetic manipulation can also be concerned. Transient transfection into donor cells of therapeutic miRNA could also be transferred into apoEVs successfully [68], providing another cargo loading approach (Table 2).

Another drug delivery strategy is loading apoEVs into materials or scaffolds with biocompatibility, not concerning the targeting property or membrane superiority, but aiming to achieve longer drug retention and controlled release. When embedded into PF-127 hydrogel, apoEVs showed the fastest skin regeneration [18] (Table 2). Although a concentration time curve is lacking in this research, a compelling retention time and promoted regeneration ability of other EVs combined with biomaterials have been provided [114–116]. In general, the combination of apoEVs and biomaterials is an attractive method with the potential for sustainable therapy.

6 Challenges, prospects, and conclusion

ApoEVs, the products, and meanwhile the information disseminators, seem to inherit the therapeutic role of apoptosis in tissue regeneration, changing death to another beginning of life. Different biogenesis pathways of apoEVs seem to be linked to cell types. To obtain information on the reason why different biogenesis patterns of apoEVs occur, investigating the functions of cells undergoing diverse apoptotic ways and respective apoEVs might be a possible direction. Moreover, morphological diversities in different biogenesis patterns of apoEVs might contribute to the clearance of apoptotic cells. For example, suppressed blebbing in Jurkat T cells caused inhibited uptake by macrophages in cell disassembly [117]. The interaction between microtubule spikes of A431 epithelial cells and phagocytes was reduced when formation of microtubule spikes was inhibited [50]. Although a conclusive explanation for such diverse patterns still needs to be clarified, diversity among the contents of apoEVs originating from different sources exists. For example, apoEVs undergoing “beads-on-string” apoptosis contain no nuclear material, unlike other groups of apoEVs [52]. In addition, research has seldom compared the underlying discrepancies in different production protocols or differences among subtypes of apoEVs. In limited literature that analyzed the content diversity of apoEVs, different attractive abilities for neutrophil were demonstrated in various groups of apoEVs. The group containing inflammatory chemokines caused secondary inflammation, which we will not anticipate to occur in tissue regeneration. This means that it is not that the more apoEVs there are, the better the regenerative effect, but the appropriate quantity and prompt processing of phagocytes, along with the optimal group of apoEVs, or the specific contents of apoEVs in another word, matter more. Figuring these puzzles out is fundamental to probe into the particular portion of apoEVs functioning directly or mediately as key therapeutics in different diseases, thus further formulating instructive protocols to study or manufacture in the future, aiming at specific pathological situations.

In conclusion, apoptosis, the silent closure of one cell, and the initiation of other cells, play pivotal roles in tissue integrity maintenance. ApoEVs may promote tissue regeneration, including tissue of skin, bone, cardiovascular system, and kidney, by activating targeted recipient cells or transferring biomolecules (DNA, RNA, and proteins) to facilitate proliferation and regulate inflammation, both locally and remotely. Nevertheless, the reason why apoEVs could be generated in different patterns in vivo, and the connection between different biogenesis patterns and the properties of respective apoEVs remain unclear. Controlling the distribution and release of biomolecules or drugs carried by apoEVs is also important for therapeutic usage, for which manipulation by engineered means and combination with biomaterials can be optional. There still is a long way to utilize the regenerative and therapeutic functions of apoEVs, many comprehensive studies need to be finished.

Funding information: This work was partially supported by the National Natural Science Foundation of China (31971251), National Key Research and Development Program of China (No. 2018YFC1106800), China National Key R&D Program
during the 13th Five-year Plan Period, West China Hospital of Sichuan University (No. ZYGD21001), Sichuan Province Science & Technology Department Projects (No. 2020YFS0082), Scientific Research Projects of Sichuan Health Commission (No. 19PJ097), The “111” Project (No. Bt6033).

Author contributions: Yixi Wang and Haider Mohammed Khan contributed equally to this work. Conceptualization: Z.Z., Z.L., and C.Z.; methodology: Y.W. and H.M.K.; validation: Y.W., Z.Z., and Z.L.; investigation: Y.W., Z.Z., Z.L., and C.Z.; visualization: X.L., Z.C., and H.M.K.; project administration: Z.Z.; funding acquisition: Z.Z., Z.L., and C.Z. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

References

[1] Tkach M, Thény C. Communication by extracellular vesicles: where we are and where we need to go. Cell. 2016;164(6):1226–32.
[2] Cheng AN, Cheng LC, Kuo CL, Lo YK, Chou HY, Chen CH, et al. Mitochondrial Lon-induced mtDNA leakage contributes to PD-1-mediated immunosuppression via STING-IFN signaling and extracellular vesicles. J Immunother Cancer. 2020;8(2):e001372.
[3] Fu Y, Sui B, Xiang L, Yan X, Wu D, Shi S, et al. Emerging understanding of apoptosis in mediating mesenchymal stem cell therapy. Cell Death Dis. 2021;12(6):596.
[4] Karpman D, Ståhl AL, Arvidsson I. Extracellular vesicles in renal disease. Nat Rev Nephrol. 2017;13(9):545–62.
[5] Poon IKH, Parkes MAF, Jiang L, Atkin-Smith GK, Tixeira R, Gregory CD, et al. Moving beyond size and phosphatidylserine exposure: evidence for a diversity of apoptotic cell-derived extracellular vesicles in vitro. J Extracell Vesicles. 2019;8(1):1608786.
[6] Pavlyukov MS, Yu H, Bastola S, Minata M, Shender VO, Lee Y, et al. Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors. Cancer Cell. 2018;34(1):119–35.e10.
[7] Gao Y, Zhang H, Zhou N, Xu P, Wang J, Gao Y, et al. Methotrexate-loaded tumour-cell-derived microvesicles can relieve biliary obstruction in patients with extrahepatic cholangiocarcinoma. Nat Biomed Eng. 2020;4(7):743–53.
[8] Atkin-Smith GK, Poon IKH. Disassembly of the dying: mechanisms and functions. Trends Cell Biol. 2017;27(2):151–62.
[9] Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol. 2008;9(3):231–41.
[10] King KL, Cidlowski JA. Cell cycle regulation and apoptosis. Annu Rev Physiol. 1998;60:601–17.
[11] Diwanji N, Bergmann A. An unexpected friend - ROS in apoptosis-induced compensatory proliferation: Implications for regeneration and cancer. Semin Cell Dev Biol. 2018;80:74–82.
[12] Fogarty CE, Diwanji N, Lindblad JL, Tare M, Amcheslavsky A, Makhijani K, et al. Extracellular reactive oxygen species drive apoptosis-induced proliferation via drosophila macrophages. Curr Biol. 2016;26(5):575–84.
[13] Li F, Huang Q, Chen J, Peng Y, Roop DR, Bedford JS, et al. Apoptotic cells activate the “phoenix rising” pathway to promote wound healing and tissue regeneration. Sci Signal. 2010;3(110):ra13.
[14] Samos J, García-Olmo DC, Picazo MG, Rubio-Vitaller A, García-Olmo D. Circulating nucleic acids in plasma/serum and tumor progression: are apoptotic bodies involved? An experimental study in a rat cancer model. Ann N Y Acad Sci. 2006;1075:165–73.
[15] Liu H, Liu S, Qiu X, Yang X, Bao L, Pu F, et al. Donor MSCs release apoptotic bodies to improve myocardial infarction via autophagy regulation in recipient cells. Autophagy. 2020;16(12):2140–55.
[16] Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep. 2019;39(1):BSR20180992.
[17] Battistelli M, Falcieri E. Apoptotic bodies: particular extracellular vesicles involved in intercellular communication. Biology (Basel). 2020;9(1):21.
[18] Liu J, Qiu X, Lv Y, Zheng C, Dong Y, Dou G, et al. Apoptotic bodies derived from mesenchymal stem cells promote cutaneous wound healing via regulating the functions of macrophages. Stem Cell Res Ther. 2020;11(1):507.
[19] Ma Q, Liang M, Limjungyawong N, Dan Y, Xing J, Li J, et al. Osteoclast-derived apoptotic bodies show extended biological effects of parental cell in promoting bone defect healing. Theranostics. 2020;10(15):6825–38.
[20] Ma Q, Liang M, Wu Y, Ding N, Duan L, Yu T, et al. Mature osteoclast-derived apoptotic bodies promote osteogenic differentiation via RANKL-mediated reverse signaling. J Biol Chem. 2019;294(29):11240–7.
[21] Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CKCL12-dependent vascular protection. Sci Signal. 2009;2(100):ra81.
[22] Bussolati B, Camussi G. Renal injury: early apoptotic extracellular vesicles in injury and repair. Nat Rev Nephrol. 2017;13(9):523–4.
[23] Dou G, Tian R, Liu X, Yuan P, Ye Q, Liu J, et al. Chimeric apoptotic bodies functionalized with natural membrane and modular delivery system for inflammation modulation. Sci Adv. 2020;6(30):eaaz2987.
[24] Bose RJC, Tharmalingam N, García Marques FJ, Sukumar UK, Natarajan A, Zeng Y, et al. Reconstructed apoptotic bodies as "nano decoys" to treat intracellular bacterial infections within macrophages and cancer cells. ACS Nano. 2020;14(5):5818–35.
[25] Caruso S, Poon IKH. Apoptotic cell-derived extracellular vesicles: more than just debris. Front Immunol. 2018;9:1486.
[26] Arienti S, Barth ND, Doward DA, Rossi AG, Dransfield I. Regulation of apoptotic cell clearance during resolution of inflammation. Front Pharmacol. 2019;10:891.

[27] Grant LR, Milic I, Devitt A. Apoptotic cell-derived extracellular vesicles: structure-function relationships. Biochem Soc Trans. 2019;47(2):509–16.

[28] Muhsin-Sharafaldine MR, McLellan AD. Tumor-derived apoptotic vesicles: with death they do part. Front Immunol. 2018;9:957.

[29] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972;26(4):239–57.

[30] Fuchs Y, Steller H. Live to die another way: modes of programmed cell death and the signals emanating from dying cells. Nat Rev Mol Cell Biol. 2015;16(6):329–44.

[31] Li M, Liao L, Tian W. Extracellular vesicles derived from apoptotic cells: an essential link between death and regeneration. Front Cell Dev Biol. 2020;8:573511.

[32] Zhang Y, Chen X, Gueydan C, Han J. Plasma membrane changes during programmed cell deaths. Cell Res. 2018;28(11):9–21.

[33] Hounsell C, Fan Y. The duality of caspases in cancer, as told by LIM kinases. Cell Death Diag. 2015;19(8):87.e4.

[34] Sharafaldine MR, McLellan AD. Tumor anti-apoptotic functions of caspases. Cell Death Different. 2016;23(7):1168–74.

[35] Moss DK, Betin VM, Malesinski SD, Lane JD. A novel role for myosin light chain kinase activity. Clin Exp Immunol. 1998;114(2):304–9.

[36] Fagury CE, Bergmann A. Killers creating new life: caspases induce apoptosis-induced proliferation in tissue repair and disease. Cell Death Differ. 2017;24(8):1390–400.

[37] Kondo S, Senoo-Matsuda N, Hiromi Y, Miura M. DRONC coordinates cell death and compensatory proliferation. Mol Cell Biol. 2006;26(19):7258–68.

[38] Wells BS, Yoshida E, Johnston LA. Compensatory proliferation in Drosophila imaginal discs requires Drön-dependent p53 activity. Curr Biol. 2006;16(16):1606–15.

[39] Fan Y, Bergmann A. Distinct mechanisms of apoptosis-induced compensatory proliferation in proliferating and differentiating tissues in the Drosophila eye. Dev Cell. 2008;14(4):399–410.

[40] Love NR, Chen Y, Ishibashi S, Kritslikguok P, Lea R, Koh Y, et al. Apoptosis-induced reactive oxygen species are required for successful Xenopus tadpole tail regeneration. Nat Cell Biol. 2013;15(2):222–8.

[41] Chera S, Ghila L, Dobretz K, Wenger Y, Bauer C, Buzgariu W, et al. Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. Dev Cell. 2009;17(2):279–89.

[42] Yosefzon Y, Soteriou D, Feldman A, Kostic L, Brown S, et al. Caspase-3 regulates Yap-dependent cell proliferation and organ size. Mol Cell. 2018;70(4):573–87.e4.

[43] Fujita J, Crane AM, Souza MK, Dejosez M, Kyba M, Flavell RA, et al. Caspase activity mediates the differentiation of embryonic stem cells. Cell Stem Cell. 2008;2(6):595–601.

[44] Miura M, Chen XD, Allen MR, Bi Y, Gronthos S, Seo BM, et al. A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. J Clin Invest. 2004;114(12):1704–13.

[45] Liu D, Kou X, Chen C, Liu S, Liu Y, Yu W, et al. Circulating apoptotic bodies maintain mesenchymal stem cell homeostasis and ameliorate osteopenia via transferring multiple cellular factors. Cell Res. 2018;28(9):918–33.

[46] Théry C, Witwer KW, Akawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):153750.

[47] Baxter AA. Stoking the fire: how dying cells propagate inflammatory signalling through extracellular vesicle trafficking. Int J Mol Sci. 2020;21(19):7256.

[48] Zilzingl M, Fürnrohr J, Janko C, Munoz LE, Voll RE, Gregory CD, et al. Loading of nuclear autoantigens prototypically recognized by systemic lupus erythematosus sera into late apoptotic vesicles requires intact microtubules and myosin light chain kinase activity. Clin Exp Immunol. 2015;179(1):39–49.

[49] Arber S, Barbayannis FA, Hanser H, Schneider C, Stanyon CA, Bernard O, et al. Regulation of actin dynamics through phosphorylation of cofillin by LIM-kinase. Nature. 1998;393(6687):805–9.

[50] Atkin-Smith GK, Tixeira R, Paone S, Mathivahan S, Collins C, Liem M, et al. A novel mechanism of generating extracellular vesicles during apoptosis via a beads-on-a-string membrane structure. Nat Commun. 2015;6:7439.

[51] Smrzka P, Greenberg PD, Park J, Lee C, Yen MT, et al. A novel mechanism of generating extracellular vesicles 2018. Cell Death Different. 2015;22(1):91–98.

[52] Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, et al. Pannexin 1 channels mediate apoptotic cell sensing, recognition, engulfment, and digestion. Cold Spring Harb Perspect Biol. 2013;5(4):a019526.

[53] Ho A, Zou Y, Zhou X, Maelicke A, Harper JW, et al. Novel role for heat shock protein 90 in medicine. Cell. 2003;115(7):903–15.

[54] Dieudé M, Bell C, Turgeon J, Beillevaire D, Pomerleau L, Yang B, et al. The 205 proteasome core, active within apoptotic exosome-like vesicles, induces autoantibody production and accelerates rejection. Sci Transl Med. 2015;7(318):318ra200.

[55] Fanger P, Kallman RJ, Benirschke K. Non-complementary sequences: their possible role in the relationship between apoptosis and tumourigenesis. J Cell Physiol. 1994;158(2):237–40.

[56] Torr EE, Gardner DH, Thomas L, Goodall DM, Bielemeier A, Willetts R, et al. Apoptotic cell-derived ICAM-3 promotes both macrophage chemotraction to and tethering of apoptotic cells. Cell Death Differ. 2012;19(4):671–9.

[57] Eguchi A, Mulya A, Lazic M, Radhakrishnan D, Berk MP, Povero D, et al. Microparticles release by adipocytes act as
Apoptotic cells derived micro/nano-sized EVs in tissue regeneration

“find-me” signals to promote macrophage migration. PLoS One. 2015;10(4):e0123310.

60. Berda-Haddad Y, Robert S, Salers P, Zekraoui L, Farnarier C, Dinarello CA, et al. Sterile inflammation of endothelial cell-derived apoptotic bodies is mediated by interleukin-1α. Proc Natl Acad Sci U S A. 2011;108(51):20684–9.

61. Holmgren L, Szeles A, Rajnavölgyi E, Folkman J, Klein G, Emborg I, et al. Horizontal transfer of DNA by the uptake of apoptotic bodies. Blood. 1999;93(11):3956–63.

62. Halicka HD, Bedner E, Darzynkiewicz Z. Segregation of RNA and separate packaging of DNA and RNA in apoptotic bodies during apoptosis. Exp Cell Res. 2000;260(2):248–56.

63. Zhu Z, Zhang D, Lee H, Wu J, Hu K, et al. Macrophage-derived apoptotic bodies promote the proliferation of the recipient cells via shuttling microRNA-221/222. J Leukoc Biol. 2017;101(6):1349–59.

64. Hardy MP, Audemard É, Migneault F, Feghaly A, Brochu S, Gendron P, et al. Apoptotic endothelial cells release small extracellular vesicles loaded with immunostimulatory viral-like RNAs. Sci Rep. 2019;9(1):7203.

65. Lleo A, Zhang W, McDonald WH, Seeley EH, Leung PS, Coppel RL, et al. Shotgun proteomics: identification of unique protein profiles of apoptotic bodies from biliary epithelial cells. Hepatology. 2014;60(4):1314–23.

66. Turiák L, Misják P, Szabó TG, Aradi B, Pálóczi K, Ozohanics O, et al. Proteomic characterization of thyocyme-derived microvesicles and apoptotic bodies in BALB/c mice. J Proteomics. 2011;74(10):2025–33.

67. García-Pastor C, Blázquez-Serra R, Bosch RJ, Lucio Cazaña FJ, Fernández-Martínez AB. Apoptosis and cell proliferation in proximal tubular cells exposed to apoptotic bodies. Novel pathophysiological implications in cisplatin-induced renal injury. Biochim Biophys Acta Mol Basis Dis. 2019;1865(9):2504–15.

68. Zheng C, Sui B, Zhang X, Hu J, Chen J, Liu J, et al. Apoptotic vesicles restore liver macrophage homeostasis to counteract type 2 diabetes. J Extracell Vesicles. 2021;10(7):e12109.

69. Schwarz N, Tumpara S, Wrenger S, Ercetin E, Hamacher J, Welte T, et al. Alpha1-antitrypsin protects lung cancer cells from staurosporine-induced apoptosis: the role of bacterial lipopolysaccharide. Sci Rep. 2020;10(1):9563.

70. Ding Y, Wang B, Chen X, Zhou Y, Ge J. Staurosporine suppresses survival of HepG2 cancer cells through Omi/HtrA2-mediated inhibition of PI3K/Akt signaling pathway. Tumour Biol. 2017;39(3):1030428317694317.

71. Huang Y, Fu Z, Dong W, Zhang Z, Mu J, Zhang J. Serum starvation-induced downregulation of Bcl-2/Bax confers apoptosis in tongue coating-related cells in vitro. Mol Med Rep. 2018;17(4):5057–64.

72. Shen G, Krienke S, Schiller P, Nießan A, Neu S, Eckstein V, et al. Microvesicles released by apoptotic human neutrophils suppress proliferation and IL-2/IL-2 receptor expression of resting T helper cells. Eur J Immunol. 2017;47(5):900–10.

73. Chen H, Kasagi S, Chia C, Zhang D, Tu E, Wu R, et al. Extracellular vesicles from apoptotic cells promote TGFβ production in macrophages and suppress experimental colitis. Sci Rep. 2019;9(1):5875.

74. Ding N, Liu C, Yao L, Bai Y, Cheng P, Li Z, et al. Alendronate induces osteoclast precursor apoptosis via peroxisomal dysfunction mediated ER stress. J Cell Physiol. 2018;233(9):7415–23.

75. Brock CK, Wallin ST, Ruiz OE, Samms KM, Mandal A, Sumner EA, et al. Stem cell proliferation is induced by apoptotic bodies from dying cells during epithelial tissue maintenance. Nat Commun. 2019;10(1):1044.

76. Eisenhofer GT, Lofthus PD, Yoshigi M, Otsuna H, Chien CB, Morcos PA, et al. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. Nature. 2012;484(7395):546–9.

77. Fan Y, Bergmann A. Apoptosis-induced compensatory proliferation. The Cell is dead. Long live the Cell! Trends Cell Biol. 2008;18(10):467–73.

78. Gupta KH, Goldufsky JW, Wood SJ, Tardi NJ, Moomty GS, Gilbert DZ, et al. Apoptosis and compensatory proliferation signaling are coupled by CrkI-containing microvesicles. Dev Cell. 2017;41(6):674–84.e5.

79. Shafikhani SH, Mostov K, Engel J. Focal adhesion components are essential for mammalian cell cytokinesis. Cell Cycle. 2008;7(18):2868–76.

80. Shafikhani SH, Engel J. Pseudomonas aeruginosa type III-secreted toxin ExoS inhibits host-cell division by targeting cytokinetics at multiple steps. Proc Natl Acad Sci U S A. 2006;103(42):15605–10.

81. Przekora A. A concise review on tissue engineered artificial skin grafts for chronic wound treatment: can we reconstruct functional skin tissue in vitro? Cells. 2020;9(7):1622.

82. Howard EE, Pasiakos SM, Blessos CN, Fussell MA, Rodriguez NR. Divergent roles of inflammation in skeletal muscle recovery from injury. Front Physiol. 2020;11:e87.

83. Harrell CR, Markovic BS, Fellbaum C, Arsenijevic A, Volarevic V. Mesenchymal stem cell-based therapy of osteoarthritis: Current knowledge and future perspectives. Biomed Pharmacother. 2019;109:2318–26.

84. Mountzias PM, Spicer PP, Kasper FK, Mikos AG. Harnessing and modulating inflammation in strategies for bone regeneration. Tissue Eng Part B Rev. 2011;17(6):393–402.

85. Liu S, Jiang L, Li H, Shi H, Luo H, Zhang Y, et al. Mesenchymal stem cells prevent hypertrophic scar formation via inflammatory regulation when undergoing apoptosis. J Invest Dermatol. 2014;134(10):2648–57.

86. Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-kB signaling in resident macrophages. Blood. 2011;118(2):330–8.

87. Qi Y, Jiang D, Sindrilaru A, Schatz S, Treiber N, et al. Microvesicles derived from apoptotic bodies induce apoptosis of liver macrophages. J Cell Physiol. 2017;233(1):26–38.

88. Wu Y, Zhang Y, Bai L, Wang Q, Xue L, Su Z, et al. An apoptotic body-biomimetic liposome in situ upregulates anti-inflammatory macrophages for stabilization of atherosclerotic plaques. J Control Release. 2019;316:236–49.

89. Chen H, Lin KC, Wallace CG, Chen YT, Yang CC, Leu S, et al. Additional benefit of combined therapy with melatonin and apoptotic adipose-derived mesenchymal stem cell against sepsis-induced kidney injury. J Pineal Res. 2014;57(1):16–32.
[90] Tyukavin AI, Belostotskaya GB, Zakharov E, Ivinke D, Rad’kova SV, Knyazev NA, et al. Apoptotic bodies of cardiomyocytes and fibroblasts - regulators of directed differentiation of heart stem cells. Bull Exp Biol Med. 2020;170(1):112–7.

[91] Tyukavin AI, Belostotskaya GB, Golovanova TA, Galagudza MM, Zakharov EA, Burkova NV, et al. Stimulation of proliferation and differentiation of rat resident myocardial cells with apoptotic bodies of cardiomyocytes. Bull Exp Biol Med. 2015;159(1):138–41.

[92] Kou X, Xu X, Chen C, Sanmillan ML, Cai T, Zhou Y, et al. The Yixi Wang et al. The biocorona: a challenge for the biomedical application of nanoparticles. Nanotechnol Rev. 2017;6(4):345–53.

[93] Gajbhiye KR, Pawar A, Mahadik KR, Gajbhiye V. PEGylated nanocarriers: a promising tool for targeted delivery to the brain. Colloids Surf B Biointerfaces. 2020;187:110770.

[94] Wang Q, Ren Y, Mu J, Egilmez NK, Zhuang X, Deng Z, et al. Grapefruit-derived nanovectors use an activated leukocyte trafficking pathway to deliver therapeutic agents to inflammatory tumor sites. Cancer Res. 2015;75(12):2520–9.

[95] Jin K, Luo Z, Zhang B, Pang Z. Biomimetic nanoparticles for inflammation targeting. Acta Pharm Sin B. 2018;8(1):23–33.

[96] Johnson P, Ruffell B. CD44 and its role in inflammation and inflammatory diseases. Inflamm Allergy Drug Targets. 2009;8(3):208–20.

[97] Li T, Shi S, Goel S, Shen X, Xie X, Chen Z, et al. Recent advances in mesoporous silica nanoparticles towards therapeutic applications for cancer. Acta Biomater. 2019;89:1–13.

[98] Castillo RR, Lozano D, González B, Manzano M, Izquierdo-Barba I, Vallet-Regí M. Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery: an update. Expert Opin Drug Deliv. 2019;16(4):415–39.

[99] Huang CC, Kang M, Shirazi S, Lu Y, Cooper LF, Gajendrareddy P, et al. 3D encapsulation and tethering of functionally engineered extracellular vesicles to hydrogels. Acta Biomater. 2021;126:199–210.

[100] Tao SC, Guo SC, Li M, Ke QF, Guo YP, Zhang CQ. Chitosan wound dressings incorporating exosomes derived from microRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full-thickness skin defects in a diabetic rat model. Stem Cells Transl Med. 2017;6(3):736–47.

[101] Cunnane EM, Lorentz KL, Ramaswamy AK, Gupta P, Mandal BB, O’Brian FJ, et al. Extracellular vesicles enhance the remodeling of cell-free silk vascular scaffolds in rat aorta. ACS Appl Mater Interfaces. 2020;12(24):26955–65.

[102] Wilasp E, Uthaisang W, Elnström-Magnusson C, Hanayama R, Tanaka M, Nagata S, et al. Bridge over troubled water: milk fat globule epidermal growth factor 8 promotes human monocyte-derived macrophage clearance of non-blebbing phosphatidylserine-positive target cells. Cell Death Differ. 2007;14(5):1063–5.

[103] Hristov M, Erl W, Linder S, Weber PC. Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells in vitro. Blood. 2004;104(9):2761–6.