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REVIEW

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Tailoring of apoptotic bodies for diagnostic and therapeutic applications:advances, challenges, and prospects

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Abstract

Apoptotic bodies (ABs) are extracellular vesicles released during apoptosis and possess diverse biological activities. Initially, ABs were regarded as garbage bags with the main function of apoptotic cell clearance. Recent research has found that ABs carry and deliver various biological agents and are taken by surrounding and distant cells, affecting cell functions and behavior. ABs-mediated intercellular communications are involved in various physiological processes including anti-inflammation and tissue regeneration as well as the pathogenesis of a variety of diseases including cancer, cardiovascular diseases, neurodegeneration, and inflammatory diseases. ABs in biological fluids can be used as a window of altered cellular and tissue states which can be applied in the diagnosis and prognosis of various diseases. The structural and constituent versatility of ABs provides flexibility for tailoring ABs according to disease diagnostic and therapeutic needs. An in-depth understanding of ABs' constituents and biological functions is mandatory for the effective tailoring of ABs including modification of bio membrane and cargo constituents. ABs' tailoring approaches including physical, chemical, biological, and genetic have been proposed for bench-to-bed translation in disease diagnosis, prognosis, and therapy. This review summarizes the updates on ABs tailoring approaches, discusses the existing challenges, and speculates the prospects for effective diagnostic and therapeutic applications.

Highlights

- Apoptotic bodies (ABs), a type of cellular waste product discovered as a biomaterial with therapeutic potential
- ABs mediated intercellular communication, including the transmission of biological signals and biological substances
- Apoptotic bodies exhibit significant research and application potential in the prevention, diagnosis, and treatment of diseases.
- The functionality of apoptotic bodies can be enhanced through drug loading, surface modification, and biomimetic preparation.

Keywords Apoptotic bodies, Engineering method, Biology function

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Introduction

Apoptotic bodies (ABs) are small membrane-bound vesicles derived from apoptotic cells, which have long been recognized for their critical role in the maintenance of tissue homeostasis and the elimination of unwanted or damaged cells. ABs are formed by programmed cell death, namely apoptosis, which is essential for the growth, development, tissue renewal, and homeostasis of organisms [1, 2]. Unlike necrosis, which is a form of cell death that releases cellular contents and triggers an inflammatory response, apoptosis is a controlled process that eliminates individual cells without triggering inflammation or damage to the surrounding tissues [3]. ABs were first discovered by researchers in 1962–1964 [4]. Initially, ABs were considered cellular waste produced by cell damage, without any specific physiological function [5]. Studies in the last decade found the diagnostic and therapeutic potential of ABs. Recent studies have shown that ABs are not only involved in the clearance of apoptotic cells but also have unique biological properties that make ABs ideal candidates for disease diagnosis and therapy [2, 6–12]. ABs carry diverse cargo such as proteins, metabolites, and nucleic acids that can be transferred to adjacent cells, thereby regulating cellular function, or signaling pathways to promote healing and tissue regeneration [7, 13–21]. Moreover, ABs from specific cell types have been shown to alleviate inflammation [15, 22], disease progression [9, 10], and tissue injury [11, 23], suggesting their potential utility in disease management. Advancements in proteomics, transcriptomics, and cellular engineering have made it possible to identify novel molecules and pathways involved in ABs biology, construct ABs with good biocompatibility and diverse functions, and expand their application in disease diagnosis and therapy [24]. Although ABs have great application potential in disease diagnosis and treatment, the exploitation of ABs is still in its infancy, and several challenges need to be addressed, including the optimization of ABs isolation and characterization techniques, the standardization of experimental protocols, and the development of innovative therapeutic strategies based on ABs tailoring.

Based on the abovementioned facts, this review aims to provide readers with a comprehensive understanding of ABs' tailoring for use in disease diagnosis and therapy. The review begins by presenting an overview of ABs biology, including their origin, occurrence, and recent developments. Then, it explores the biological functions and potential applications of ABs in disease diagnosis and therapy followed by a discussion on the typing, induction, and engineering modification of ABs. Finally, the review summarizes the advances in ABs-related research and prospects for future research in the field of ABs' tailoring for application in disease diagnosis and therapy. The information presented in this review article will be valuable to researchers and clinicians working in the field of AB-based disease diagnosis, management, and therapy.

Methods

We conducted literature searches on three databases, including Web of Science, PubMed, and Scopus in January 2023. The search period ranged from 1st January 2010 to 1st January 2023. The search strategies used were as follows. Web of Science: (TS = ("apoptotic bodies*" OR "apoptotic body*" OR "extracellular vesicle*")); PubMed: (apoptotic bodies* OR apoptotic body* OR extracellular vesicle*); and Scopus: TITLE-ABS-KEY ("apoptotic bodies*" OR "apoptotic body*" OR "extracellular vesicle*").We also conducted a manual search by reviewing the reference lists of key published articles to identify additional eligible studies.

The included articles were: (1) peer-reviewed original research article or review article; (2) published in English. The excluded articles were: (1) letter, commentary, proceeding paper, conference abstract, meta-analysis, patent, case report, editorial, book chapter, non-peer-reviewed publication (including preprint), or retracted publication; (2) published in languages other than English.

The biological features of ABs

Based on size and biogenesis, extracellular vesicles (EVs) can be classified into three subtypes: exosomes (30–150 nm), microvesicles (50–1000 nm), and ABs (50–5000 nm) [25, 26]. The distinctions among these EVs are listed in Table 1. Different from exosomes and microvesicles, ABs are membrane-bound vesicles that are released by dying cells in the final stage of apoptosis [27]. This controlled procedure consists of three steps (Fig. 1) [28]. Firstly, apoptotic membrane blebbing is a crucial step in ABs formation mediated by cysteine protease 3 (caspase-3) phosphorylation and activation of protein kinases, particularly Rho-associated protein kinase 1 (ROCK1) [29, 30]. Other kinases such as myosin light chain kinase (MLCK), Lim domain kinase 1 (LIMK1), and p21-activated kinase (PAK2) are non-essential kinases but may also be involved in apoptotic membrane blebbing [29]. These kinases contribute to actomyosin cortex contraction, leading to the generation of hydrostatic pressure and the entry of intracellular fluid. These processes cause the separated membrane to expand [30, 31]. After expansion, the actin and myosin II gathered in the vesicles to form a cortex attached to the plasma membrane, causing the expansion rate to slow down [30]. The contraction and assembly of this cortex ultimately

Table 1 Comparisons of ABs, exosomes, and microvesicles' biological properties

	ABs	Exosomes	Microvesicles	Refs.
Size (diameter)	50–5000 nm	30–150 nm	50–1000 nm	[25, 26]
Morphology	Heterogeneous	Cup-shaped	Various shapes	[25, 26]
Lipid composition	High PS exposure and cholesterol	LBPA, low PS exposure, cholesterol, ceramide, contains lipid rafts, and sphingomyelin	High PS exposure and cholesterol	[25, 26]
Biogenesis mechanism	Cell shrinkage and death	Exocytosis of intracellular multivesicular bodies	Plasma membrane shedding	[51–53]
Mode of extracellular release	Regulated	Constitutive and regulated	Regulated	[51–53]
Characteristic biomarkers	PTPRC, Caspase-3, C3b, C1q, CRT, TSP, H_2AX, LFA-1, CD45, and PS	Alix, TSG101, HSP70, HSP90, CD9, CD63, CD81, Annexin I, Annexin II, Annexin V, Flotllin-1, Flotillin-2, ICAM, TSP1, and periostin	Selectins, integrins, CD40, metalloproteinases, and PS	[33, 53, 54]
Content	Protein, nucleic acids (DNA, miRNA, mRNA, non-coding RNA), lipid, metabolite, residual cytoplasm, and organelles	Protein, nucleic acids (DNA, miRNA, mRNA), lipid, and metabolite	Protein, nucleic acids (DNA, miRNA, mRNA), lipid, and metabolite	[25, 26]

ABs apoptotic bodies, *PS* phosphatidylserine, *LSBPA* 2,2′-lysobisphosphatidic acid, *PTPRC* protein tyrosine phosphatase receptor C, *Caspase-3* cysteine protease 3, *CRT* calreticulin, *TSP* thrombospondin, *LFA-1* lymphocyte function-associated antigen 1, *TSG* tumor susceptibility gene 101, heat shock protein 70, *ICAM* intercellular adhesion molecules, *TSP1* thrombospondin 1

result in the formation of plasma membrane vesicles [30]. Apoptotic membrane protrusions generated by apoptotic cells are methods independent of apoptotic membrane blebbing to form ABs [32, 33]. There are three kinds of membrane protrusions: microtubule spikes inhibited by pan-catenin 1 (PANX1) channel and activated by vesicle transport [34, 35], beaded protrusions activated by transmembrane receptor plexus B2 (PlexB2) and microtubules [33, 35], and PANX1-inhibited apoptopodia [35, 36]. Finally, the fragmentation of membrane protrusion forms independent ABs [32, 33].

As cell debris, ABs inherit cellular components and surface markers from parent cells (Fig. 2) [37]. The content of ABs can be utilized for their isolation, characterization, and diagnostic and therapeutic applications. Proteins and non-protein surface markers can be utilized in ABs isolation and biological signals. Protein markers including thrombospondin (TSP) [10, 14], complement proteins C3b and C1q [38], leukocyte common antigen protein tyrosine phosphatase receptor C (PTPRC) [38], integrin lymphocyte function-associated antigen 1 (LFA-1) [38], caspase-3 [8, 39], calreticulin (CRT) [40], histone H_2AX [41], and leukocyte common antigen (LCA) such as CD45 [38] are commonly utilized ABs surface markers. One of the signs of ABs is to contain nuclear proteins, which differs from other EVs [42]. Phosphatidylserine (PS) is a non-protein marker of ABs. PS is a component of cell membranes which is normally located under the cell membrane in normal cells, and during apoptosis, enzymes catalyze the externalization of PS [19, 43–48].

Credited with unique biological properties, ABs exhibit unrivaled advantages in target accuracy, therapeutic effectiveness, and safety compared to other EV-based approaches. First, benefiting from the specific recognition and efferocytosis mechanisms including "find-me" and "eat-me" signals, ABs exhibit fewer off-target effects and more potent responses compared to EVs derived from non-apoptotic cells. Furthermore, among all osteoclast-derived EVs, ABs show the highest level of nuclear factor kappa B (RANK), granting them the greatest osteogenic potency [19]. Additionally, inherited from parent cells, ABs do not contain autoantibodies [49], which offers advantages in regenerative therapy, especially in organ transplantation, since they do not induce inflammatory responses or graft rejection as observed in other EVs.

Although various biological features make ABs a powerful candidate in disease diagnosis and therapy, the complexity of their constituents causes ambiguity in characterization [50]. Therefore, further improvement in ABs' isolation and characterization is required to fully utilize their superior potential in clinical translation.

Isolation and characterization of ABs

During apoptosis, cells form ABs through various means, which differ in composition, size, and other biology properties [32]. Therefore, accurate isolation and characterization of various ABs is essential for their effective application in disease diagnosis and therapy. Among the most used methods for ABs isolation are differential centrifugation, filtration, and fluorescence-activated cell sorting (FACS). We summarize these three separation

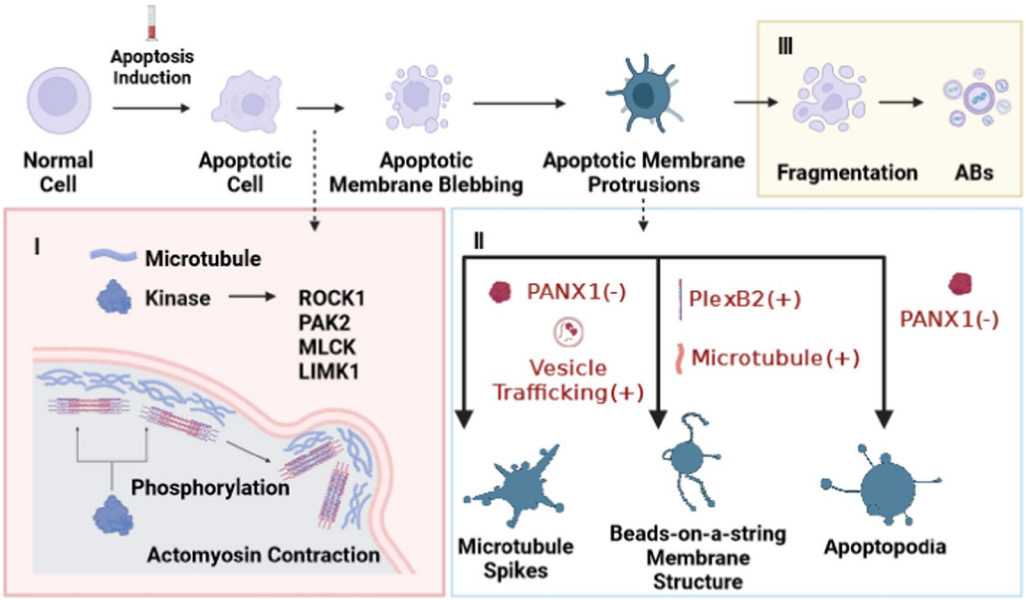

Fig. 1 Illustration of the mechanism of ABs biogenesis. (I) Cell membrane blebbing is activated by active cysteine protease 3 to activate ROCK1, PAK2, MLCK, and LIMK1, leading to actomyosin contraction. (II) Apoptotic membrane protrusion formation through the PANX1 channels and vesicle transport, resulting in the formation of microtubule spikes, bead-like protrusions, and apoptopodia. (III) Fragmentation to form ABs. *LIMK1* lim domain kinase 1, *MLCK* myosin light chain kinase, *PAK2* p21-activated kinase, *PANX1* pan-catenin 1, *PlexB2* plexus B2, *ROCK1* Rho-associated protein kinase 1. Created with BioRender.com

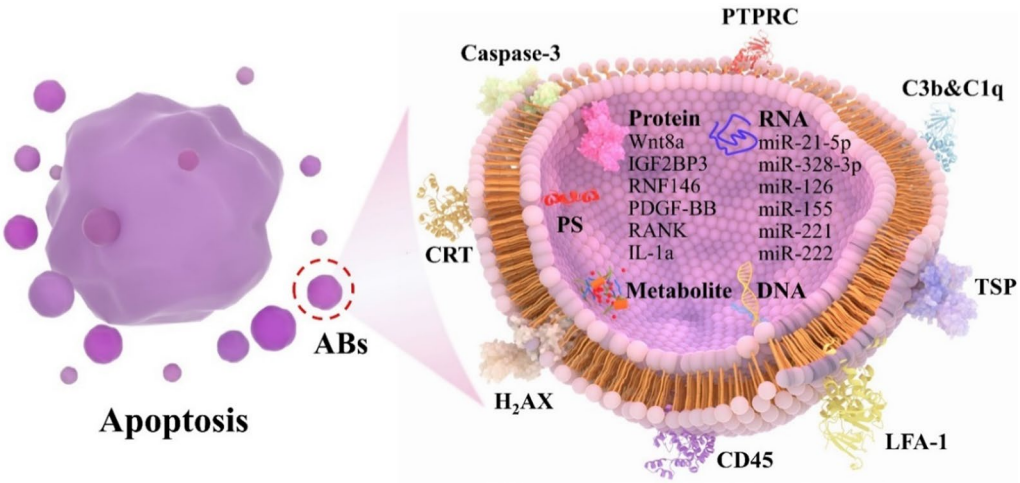

Fig. 2 The surface markers and bioactive cargos of ABs. *CRT* calreticulin, *PS* phosphatidylserine, *PTPRC* protein tyrosine phosphatase receptor C, *TSP* thrombospondin, *LFA-1* lymphocyte function-associated antigen 1

methods in detail below, including their separation principles, main applications, and advantages as well as disadvantages (Table 2). The characterization methods of ABs are summarized based on their features including specific markers and physicochemical properties (Table 3). These mainly include flow cytometry (FCM), immunohistochemistry, low-temperature transmission electron microscopy (Cryo-TEM), nanoparticle tracking technology (NTA), and nanopore technology [55–58].

There is currently no standardized method for the isolation and characterization of each subpopulation of ABs, primarily due to a lack of specific markers and overlaps in physicochemical properties. Careful selection of suitable techniques for ABs' isolation and characterization based on their application objectives is crucial.

Table 2 The isolation methods of ABs

Method	Principle	Application	Advantages	Disadvantages	Refs.
Differential centrifugation	Difference between the density of ABs and other components in the mixture	ABs extraction of single-cell type	High purity(< 83%), quick (30 min), and relatively higher yield	Low specificity toward biomarkers	[59–61]
Filtering method	Size of ABs (1–5 μm)	Combination with differential centrifugation	Simple operation	Low purity and yield	[14, 60, 62–64]
Flow cytometry	The relative size, particle size, and specific biomarkers of ABs	ABs extraction of multiple cell types	High purity(< 99%)	Complex procedure and time-consuming	[59, 65]

Most importantly, further exploration into the isolation and characterization of ABs is urgently required to fully understand the complexity of apoptosis within biological systems and provide a basis for future research on developing ABs as diagnostic and therapeutic targets.

The sources of ABs for diagnostic and therapeutic applications

ABs come from a wide variety of sources, including cells, body fluids, and tissues (Fig. 3). ABs from different sources have different functions and potential diagnostic and therapeutic applications. ABs are derived from a variety of cellular sources and exhibit distinct functions. Osteoclast-derived ABs stimulate osteogenesis and mitigate osteoporosis [9, 66]. MSC-derived ABs possess anti-inflammatory properties and facilitate tissue healing [14]. Immune cell-derived ABs exhibit remarkable anti-inflammatory properties, making them promising candidates for treating inflammatory disorders [38, 67, 68]. Endothelial cell-derived ABs induce angiogenesis and play a crucial role in wound repair [11, 23]. Cardiomyocyte-derived ABs promote myocardial regeneration and enhance myocardial infarction (MI) recovery. Recent studies demonstrated that ABs extracted from rat cardiomyocytes enhanced the proliferation and differentiation of cardiac stem cells, thereby improving myocardial contractility in rats [69, 70]. Cancer cell-derived ABs have the potential to penetrate the blood–brain barrier (BBB), offering possibilities for targeted drug delivery in the brain [71–74]. In addition, like other EVs, ABs derived from cells have the advantages of high purity and good stability [6, 14]. The low production of ABs from cells limits their large-scale development and utilization. However, with the breakthrough of rapid cell expansion technology in recent years, especially 3D cell culture technology [75–78], these limitations are radially overcome.

Unlike cell-derived ABs with high purity and stable performance, body fluid and tissue-derived ABs are more complex, contain more disease information, and change with patients' physical conditions. Therefore, body fluid-derived ABs have high diagnostic and prognostic values. The main sources of ABs from body fluids include blood, cerebrospinal fluid, urine, and other sources. Blood ABs are readily available and can be used as a non-invasive way to monitor disease activity and prognosis in various pathological conditions including cerebrovascular and neurodegenerative [79]. Urinary ABs can diagnose kidney disease and are expected to be used in clinical trials

Table 3 The characterization methods of ABs

Principle	Method	Application	Advantages	Disadvantages	Refs.
Biomarkers	FCM	PS measurement	Most commonly used	Complex procedure and lack of specific markers	[37]
	Immunohistochemistry	H_2AX characterization	High specificity	_____	[41]
Physicochemical properties	Cryo-TEM	Determination of particle size and shape	High resolution	Use of harsh conditions and hexagon/glassy ice-polluted images	[55, 56]
	NTA	Analysis of the number and size of ABs	_____	_____	[57]
	Nanopore technology	Gene sequencing, determination of particle size, and identification of cell types specific ABs	Simple procedure	Time-consuming	[58]

FCM:flow cytometry, *PS*:phosphatidylserine, *Cryo-TEM* low-temperature transmission electron microscopy, *NTA* nanoparticle tracking technology

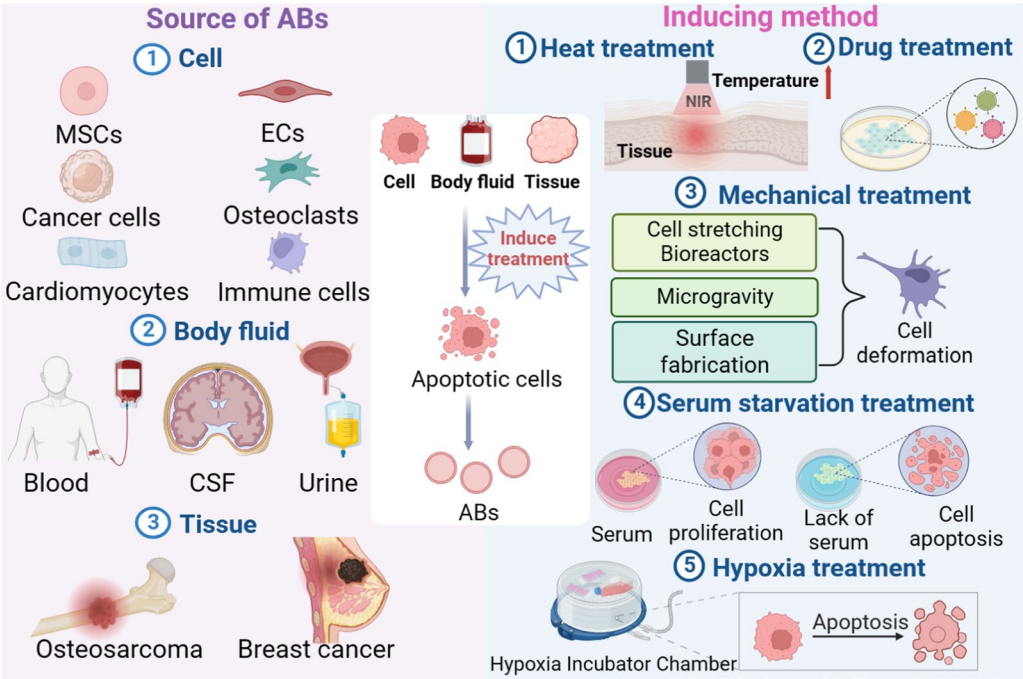

Fig. 3 The sources and induction methods of ABs. ABs can be derived from cells, body fluids, and tissues. The in vitro induction methods of ABs to improve the yield. Created with BioRender.com

[80]. Cerebrospinal fluid-derived ABs currently do not accurately reflect the degree of central nervous system disorder, and further research is needed in this direction [81]. Body fluid-derived ABs can be extracted quickly, which is conducive to clinical testing. Compared to ABs obtained from cells or body fluids, ABs isolated directly from tissues are tissue-specific and accurately reflect the tissue microenvironment. For example, the ABs index of osteosarcoma tissue combined with serum alkaline phosphatase is suggested as an effective indicator of malignancy degree and prognosis that assists in monitoring therapeutic effect [82]. Tissue-derived ABs have tissue specificity, which is more beneficial to disease diagnosis and personalized treatment.

The biological functions of ABs

In addition to a comprehensive understanding of various sources, clarifying the biological function of ABs is also the basis for their application. ABs serve as a means of intercellular communication since nearly all eukaryotic cells can release ABs that act on adjacent and distant cells [83]. Due to the complex biological information and components derived from dead cells present in ABs, they can elicit intricate and diverse effects on receiver cells [13, 14, 21]. However, there remains a lack of systematic elaboration on the biological function of ABs. This chapter aims to review the biological functions of ABs and explore their potential application prospects, including biological information transmission, biomolecule transport, and regulation of autophagy, proliferation, differentiation, and immunity.

ABs-mediated intercellular communication

ABs transmit intercellular signals and biomolecules to detect and remove necrotic cell debris (Fig. 4), which is essential for maintaining tissue homeostasis [27]. Apoptotic substances that cannot be effectively removed can induce secondary necrosis and inflammation, leading to autoimmune diseases such as systemic lupus erythematosus or other immune-related diseases [84, 85]. Therefore, understanding the intercellular communication functions of ABs, as well as their regulation mechanisms, is of great significance for the prevention and treatment of immune-related diseases. Further research on the regulatory mechanisms and interactions of these intercellular communications will guide to development of new therapeutic strategies to promote tissue regeneration and repair. ABs are involved in intercellular communication mainly via "find-me" and "eat-me" signals [86–92].

The transmission of biological signals including "find-me" and "eat-me" signals plays a crucial role in apoptotic cell clearance [27, 93]. Apoptotic cells possess substances known as "find-me" signals to attract phagocytes, followed by the quick identification and engulfment of apoptotic substances by phagocytes via "eat-me" signals. It has been observed that ABs derived

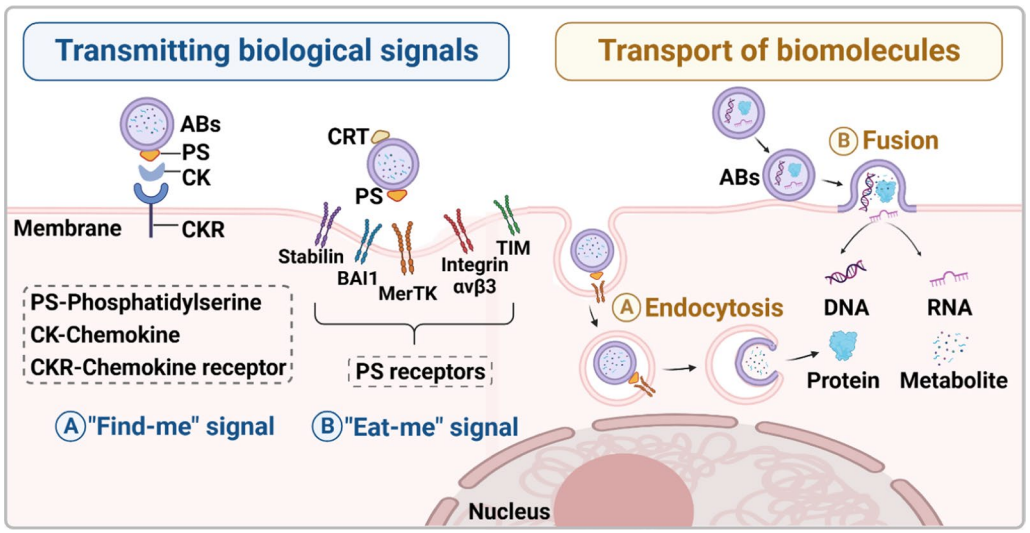

Fig. 4 Illustration of ABs involved in intercellular communication. ABs can harbor "find-me" signals to attract phagocytic cells, as well as "eat-me" signals to promote uptake by phagocytes. ABs transport biomolecules (e.g., DNA, RNA, protein, and metabolite) to neighboring cells via endocytosis and membrane fusion. *BAI1* brain-specific angiogenesis inhibitor 1, *MerTK* tyrosine-protein kinase Mer, *TIM* T cell immunoglobulin mucin proteins. Created with BioRender.com

from apoptotic cells also possess the ability to recruit phagocytes [94–96]. The "find-me" signals of ABs are PS-bound chemokines, which are released by apoptotic cells and can selectively bind to PS exposed on the surface membrane of ABs. This binding activates chemokine receptors on phagocytes via chemokines, thereby inducing phagocyte migration [87]. Subsequently, "eat-me" signals exposed on the membrane surface of ABs including PS [88–92] and CRT [40] start to exert their functions. The recognition of PS is crucial for the efficient clearance of apoptotic substances, therefore resulting in the maintenance of tissue homeostasis and prevention of autoimmunity. This process includes multiple receptors such as T cell immunoglobulin mucin proteins (TIM-1, TIM-3, TIM-4), brain-specific angiogenesis inhibitor 1 (BAI1), tyrosine-protein kinase Mer (MerTK), stabilin 1 and 2 [86, 88–92]. PS exposure on the membrane can also be recognized by integrin αvβ3 to repair cell damage and improve the osteogenic and adipogenic differentiation ability of BMSCs [14]. In addition, CRT-mediated efferocytosis of MSC-derived ABs by macrophages reduces liver macrophage infiltration and pro-inflammatory transformation, which ameliorates T2D [40]. Although multiple ABs' surface markers have been identified, further studies are needed to determine whether they can participate in cell clearance as biological signals.

During the process of apoptosis, membrane foaming and protrusion formation facilitate the redistribution of cellular components into ABs [33]. Different formation processes affect the biomolecules carried by ABs. Beaded protrusions, for instance, contribute to the generation of ABs that lack nuclear components [33]. In addition, the cleavage and activation of caspase-3 during apoptosis can affect more than 280 downstream targets, and the resulting unique cytokines, microRNAs (miRNAs), and mitotic proteins are packaged into ABs and then transported to adjacent cells [18, 97]. Subsequently, ABs act as intercellular carriers of biomolecules. ABs mainly act on receptor cells via endocytosis and membrane fusion[17–20]. ABs can transfer DNA [16, 98], RNA [14, 15] mainly miRNA including miR-328-3p, -21-5p, -126, -155, -221 and -222 [23, 67], proteins including wnt8a, insulin growth factor 2 mRNA-binding protein 3 (IGF2BP3), ring finger protein 146 (RNF146), platelet-derived growth factor-BB (PDGF-BB), receptor activator of RANK and interleukin-1a (IL-1a) [17–20], metabolites (pyridoxine and kynurenine) [21], and other signaling molecules into the target cells (Figs. 2 and 4). ABs transport genomic DNA to adjacent cells to achieve horizontal gene transfer between different cell types, leading to the occurrence of disease [16]. In the mouse xenobiotic model, ABs have been reported to be involved in regulating the circulation of distal cells, suggesting that the reuse of ABs components may be a common biological activity in the body [14]. In addition to biomolecules, ABs can also be used as carriers for transferring influenza A virus IAV to adjacent cells to achieve virus transmission between cells [99]. Through the transport of these functional biomolecules, ABs

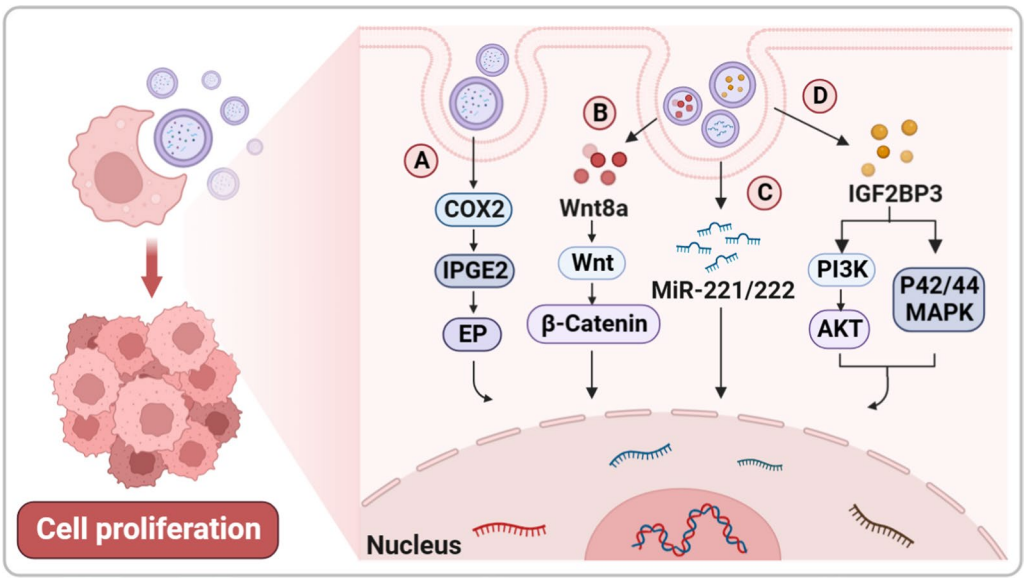

Fig. 5 Reported mechanisms of ABs regulating cell proliferation. **A** ABs promoted receptor cell proliferation through COX2/IPGE2/EP signaling pathway[17]. **B** ABs activated the Wnt/β-catenin pathway through the signal protein Wnt8a to promote cell proliferation [18]. **C** ABs induce proliferation of the recipient cells via miR-221/222 [67]. **D** ABs promoted cell proliferation by releasing IGF2BP3 and activating PI3K/AKT and P42/44 MAPK pathways in receptor cells [20]. *IGF2BP3* insulin growth factor 2 mRNA-binding protein 3. Created with BioRender.com

transmit information between cells and mediate specific biological effects.

ABs regulate cellular function

Cellular function is regulated by complex molecular mechanisms that allow cells to respond to their environment and maintain homeostasis [100]. These mechanisms involve a series of signaling pathways that regulate gene expression, protein synthesis, and cellular metabolism. ABs themselves and the biological information they carry participate in a cascade of intracellular signaling events that ultimately lead to changes in gene expression and protein synthesis. Therefore, ABs play a complex and dynamic role in the regulation of cell autophagy, proliferation, and differentiation, as well as immune responses, allowing organisms to adapt and respond to changing conditions in their environment [14, 15].

ABs regulate cell autophagy

Autophagy is a lysosomal-mediated catabolic process that maintains cellular homeostasis by processing damaged, nonfunctional, or unnecessary proteins and organelles [101]. Recent studies have revealed that ABs derived from MSCs serve the function of upregulating autophagy in receptor epithelial cells [8]. Specifically, in ECs, ABs activate lysosomal function and promote the expression of transcription factor EB (TFEB), which is the master regulator of lysosomal biogenesis and autophagy [102]. The increased presence of TFEB enhances the expression of autophagy-related genes and proteins including BECN1, LC3, and ATG5 in ECs following MI. This activated autophagy leads to increased nitric oxide (NO) production through the vascular endothelial growth factor (VEGF) signaling pathway, ultimately promoting angiogenesis in vivo. Consequently, this promotes recovery of myocardial function and improves cardiac infarction [8]. There is a complex relationship between apoptosis and autophagy, and the relatively stable state of the two is of great significance for maintaining the physiological function of cells [103]. The imbalance between apoptosis and autophagy can lead to the development of cardiovascular diseases (CVD), cancer, and brain injuries [104–106]. The critical role of ABs in regulating autophagy is expected to be involved in the regulation of these diseases.

ABs regulate cell proliferation and differentiation

Cell proliferation is the process by which cells divide and replenish aging or dead cells in vivo, which is essential for tissue regeneration [107–110]. ABs have been shown to regulate cell proliferation (Fig. 5) [17, 20]. Specifically, ABs induced by the anticancer drug cisplatin exert a proliferative effect on human renal proximal tubular HK-2 cells and induce apoptosis, subsequently giving rise to second-generation Abs [17]. Interestingly, these second-generation ABs mediate the opposite effect through the same pathway and stimulate cell proliferation [17].

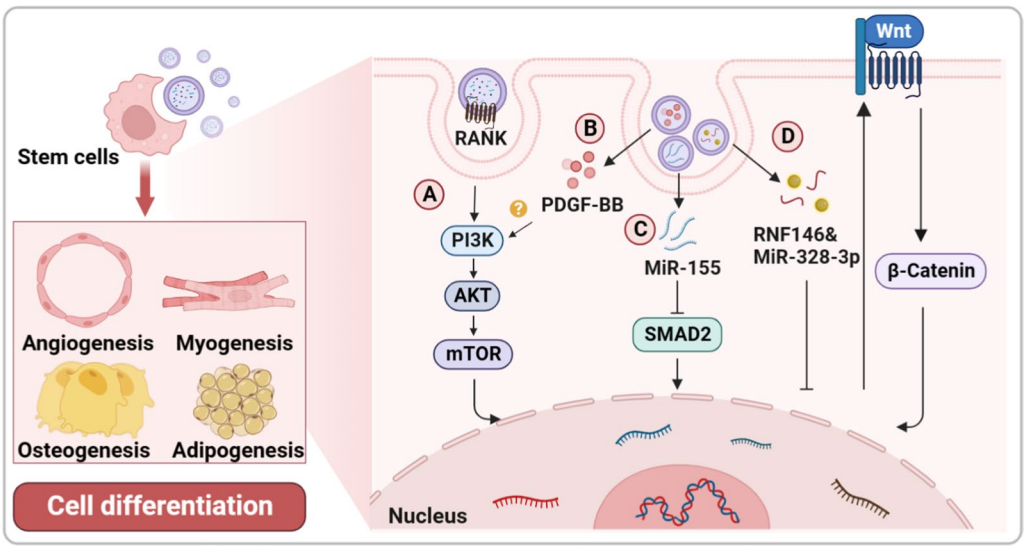

Fig. 6 Reported mechanisms of ABs regulating cell differentiation. **A** ABs promote receptor cell differentiation through the PI3K/AKT/mTOR signaling pathway [7, 9, 19]. **B** ABs carrying PDGF-BB may activate PI3K/AKT pathways to promote cell differentiation [7]. **C** ABs inhibit the SMAD2 signaling pathway via miR-155 for promoting cell differentiation [68]. **D** RNF146 and miR-328-3p in ABs inhibit the expression of gene Axin1 to activate the Wnt/β-catenin pathway, thereby promoting cell differentiation [14]. *PDGF-BB* platelet-derived growth factor-BB, *SMAD2* drosophila mothers against decapentaplegic protein 2, *RNF146* ring finger protein 146. Created with BioRender.com

Furthermore, ABs derived from tenocytes stimulate the proliferation and migration of tenocytes and bone marrow mesenchymal stem cells (BMSCs) and induce these cells to synthesize more transforming growth factor-β (TGF-β) and types I and III collagen [111]. Since TGF-β controls cell proliferation and migration and collagen is the main component of tendons, ABs promoting the high expression of the protein and collagen ultimately enhance tendon healing [111–113]. In addition, the positive role of ABs in the proliferation of fibroblasts and endothelial progenitor cells (EPCs) as well as cardiac stem cells (CSCs) is conducive to tissue regeneration, promoting angiogenesis and myocardial repair, respectively [39, 70]. However, not all proliferation promoted by ABs is benign cell growth, there is also a malignant tumor-promoting effect. ABs from tumor cells enhance programmed cell death ligand 1 (PD-L1) upregulation on GATA6 large peritoneal macrophages (GLPMs), which block cytotoxic activity of PD-1-expressing $CD8^+$ T cells and indirectly protect tumor cells [114]. ABs induced by Cytosine arabinoside (Ara-C) carry IGF2BP3 to promote blood cell proliferation and reduce apoptosis by regulating phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) and p42/44 mitogen-activated protein kinase (p42/44 MAPK) pathways [20]. Since IGF2BP3 is a potential oncogene that can induce tumorigenesis [115], ABs carrying IGF2BP3 promote oncoprotein growth in receptor cells and play a tumor-promoting role [20]. Moreover, ABs derived from macrophages exhibit the ability to suppress the expression of cyclin-dependent kinase inhibitor 1B (CDKN1B) in lung epithelial cells via miR-221 and -222, subsequently promoting epithelial cell growth, and contributing to epithelium integrity [67]. Considering that CDKN1B is a tumor suppressor [116], the inhibitory effect of ABs on CDKN1B may lead to unregulated tumor-like proliferation of epithelial cells and lung epithelium integrity [67]. In summary, on one hand, ABs promote the benign proliferation of stem cells, thereby promoting cardiovascular, tendon, and lung epithelium regeneration [111, 117], on another hand, ABs play a role in promoting abnormal cell proliferation such as tumors [20, 114].

Cell differentiation is a process whereby cells of the same types undergo morphological structure and physiological function changes, leading to the formation of distinct cell groups essential for organismal function [107, 118, 119]. Growing pieces of evidence suggest that ABs can regulate cell differentiation. The regulatory effects of ABs on cell differentiation can be categorized based on the specific direction of differentiation, including osteogenesis [14, 120], angiogenesis [7], cardiac differentiation [69, 70], and adipogenesis [14]. The regulatory mechanisms are summarized in the following (Fig. 6).

Osteogenic differentiation is a key step in bone formation, wherein BMSCs differentiate into various bone cell types, including osteogenic precursor cells, mature bone cells, and terminal bone cells. This complex process involves multicellular signal delivery

[121]. In recent years, many studies have revealed the regulatory mechanism of ABs in regulating osteogenic differentiation. For example, mOC-ABs induce the osteogenic differentiation of BMSCs and preosteoblasts through reverse signaling of the receptor activator of nuclear factor kappa B ligand (RANKL) mediated by RANK [7, 9, 19]. RANKL reverse signaling activates the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin/s6 kinase (PI3K/AKT/mTOR/S6K) signaling pathway related to osteogenesis, thereby promoting osteogenic differentiation [122]. MSCs can repeatedly use miR-328-3p and ubiquitin ligase RNF146 in ABs to inhibit the expression of Axin1, thereby activating the Wnt/β-catenin pathway, which improves the osteogenic and adipogenic abilities of MSCs [14, 120]. In addition, ABs can also inhibit bone formation. Osteocyte-derived ABs stimulate the release of tumor necrosis factor-α (TNF-α) by osteoclast precursors, which plays a key role in osteoclastogenesis and bone resorption [48, 123, 124]. MiR-155 inhibits osteogenesis but promotes adipogenesis of MSCs cultured with macrophage-derived ABs via the drosophila mothers against the decapentaplegic protein 2 (SMAD2) signaling pathway [68].

It has been reported that ABs can directly promote angiogenesis or indirectly promote vascular differentiation by recruiting endothelial stem cells to damaged vascular tissues [7, 39, 70]. ABs derived from fibroblasts and human umbilical vein endothelial cells (HUVECs) initiate the differentiation of endothelial cells (ECs) and promote angiogenesis [70, 117]. POC-ABs induce the differentiation of ECs via PDGF-BB and PI3K/AKT pathways to exert vascularization effects [7]. ABs can also indirectly exert vascularization by recruiting stem cells. ABs derived from ECs have been shown to carry miR-126 to ECs, which inhibits the function of regulator of G protein signaling 16 (RGS 16) in receptor cells, and promotes the expression of CXC chemokine CXCL12 via its receptor CXCR4 [23]. CXCL12 then recruits $CD31^+$ ECs from bone marrow to blood vessels to reduce apoptosis, which promotes atherosclerosis protection and vascular endothelial repair in mouse models. Moreover, ABs derived from retinal microvascular endothelial cells can mediate ECs releasing of angiogenic cytokines and chemokines, and induce the expression of adhesion molecules, such as intercellular adhesion molecules (ICAM), vascular cell adhesion molecules, and endothelial cell leukocyte adhesion molecule-I to recruit EPCs to damaged blood vessels and repair damaged endothelium [11]. In addition, ABs can indirectly enhance fibroblast migration by inducing macrophage M2 polarization, thus promoting angiogenesis and wound repair [39].

Myocardial regeneration refers to the process by which non-cardiac cells in the heart are converted into functioning cardiac muscle cells, called cardiomyocytes, to repair damaged or diseased heart tissue. Cardiomyocytes-derived ABs have shown the ability to stimulate the myocardial differentiation of CSCs [69, 70]. In summary, ABs have been reported to have multiple effects in regulating cell differentiation, particularly promoting osteogenesis, angiogenesis, cardiomyogenesis, and adipogenesis by the activation of specific signal pathways.

ABs mediated immune response

ABs not only participate in the regulation of autophagy and differentiation but also play a role in immune system regulation (Fig. 7). ABs can treat diseases or restore damaged functions by modulating the immune microenvironment of the body or specific diseases [15, 22, 39, 125]. Macrophages play a crucial role in the innate immune system and are important in immune regulation. Anti-inflammatory M2 macrophages are considered subtypes that inhibit osteoclast differentiation and promote bone regeneration. ABs can induce macrophages to polarize towards the anti-inflammatory M2 phenotype, mainly via the reduced expression of pro-inflammatory factors such as TNF-α, IL-6, and IL-12, and the increased expression of anti-inflammatory factors such as TGF-β and IL-10, which helps mitigate inflammatory responses [15, 39, 125]. In addition, miR-21-5p abundantly expressed in ABs targets Kruppel-like factor 6 to induce M2 polarization [15, 22].

Interestingly, ABs have also been reported to exhibit pro-inflammatory effects (Fig. 7). ABs derived from ECs containing pro-inflammatory factor IL-1α induce high expression of neutrophil chemokines such as monocyte chemoattractant protein-1 and IL-8 promoting the infiltration of neutrophils and driving sterile inflammation upon injection into the peritoneal cavity of mice, leading to tissue damage and aggravating diseases [126, 127]. To sum up, ABs mainly exert anti-inflammatory effects to promote wound healing, but it has also been reported to promote inflammation and thus play a dual role in inflammation regulation.

The application of ABs in disease diagnosis and therapy

Based on the information covered in the previous chapter, ABs possess significant functions such as participating in cell communication and transmitting biological substances. These characteristics make ABs highly promising in disease diagnosis and treatment. In this chapter, we will provide a detailed overview of the recent advancements in the application of ABs in disease diagnosis and treatment. We will explore specific cases and

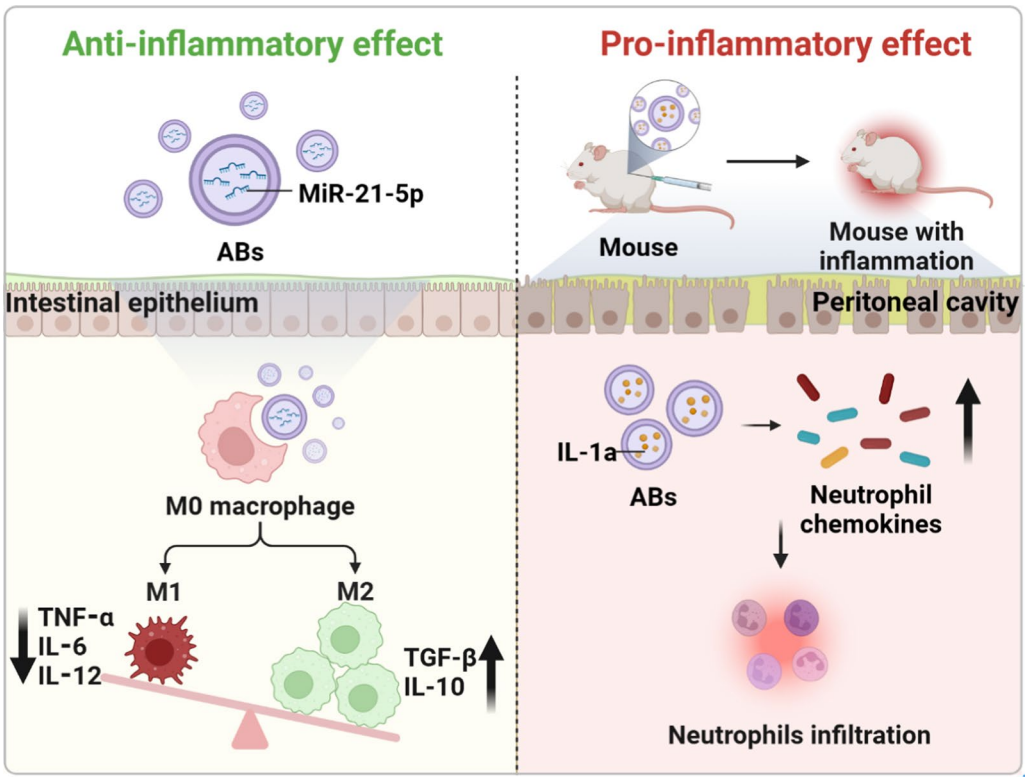

Fig. 7 ABs mediated immune response. Anti-inflammatory effect: ABs reduce the expression of pro-inflammatory factors (TNF-α, IL-6, and IL-12) in receptor macrophages, and increase the expression of anti-inflammatory factors (TGF-β and IL-10), inducing M2 macrophage polarization. Pro-inflammatory effect: ABs carry IL-1α, which can induce high expression of neutrophil chemokines and promote neutrophil infiltration to drive sterile inflammation after injection into the peritoneal cavity of mice. Created with BioRender.com

discuss how ABs have been utilized in various medical fields, including but not limited to diabetes prevention, immune disease diagnosis, bone, skin, tumor, immune disease, and cardiovascular disease treatment [128–136]. By highlighting recent research and advancements, we aim to provide a comprehensive understanding of the practical applications of ABs in the context of disease diagnosis and treatment.

Application of ABs in the prevention and diagnosis of diseases

ABs have emerged as promising clinical diagnostic biomarkers with significant implications for various aspects of human health and disease. This chapter provides a comprehensive review of the applications of ABs as biomarkers in disease prevention and diagnosis. Specifically, we delve into their role in diabetes prevention, inflammation, cancer, and cardiovascular disease [135–139].

ABs as drug candidates to prevent type 1 diabetes

Type I diabetes is an characterized by autoimmune response resulting in decreased insulin secretion and elevated blood glucose [135]. In recent studies, ABs generated by islet β cells have shown potential in preventing the development of type 1 diabetes by modulating the immune response [125]. The ABs released by dying islet β cells play a crucial role in preventing the cytotoxicity of these cells and stimulating the body's immune response as antigens to inhibit the autoimmune response [125]. Studies involving the injection of dendritic cells containing such ABs into mice have yielded promising results. These studies showed a significant reduction in the incidence of type I diabetes among the treated mice [125].

ABs as a potential diagnostic biomarker for graft rejection

In the pathology of intestinal transplants (ITx), crypt apoptosis in the intestinal epithelium plays a crucial role in diagnosing graft-versus-host disease (GVHD) and acute cellular rejection (ACR) [140]. The number of ABs observed in the crypts is used as a diagnostic feature [41, 141–143]. In normal cases of ITx, 2 or fewer ABs in 10 consecutive crypts are considered within the normal range. If there are 6 ABs, it is indicative of mild

ACR. However, when 3 to 5 ABs are present, the diagnosis becomes problematic and is often classified as indeterminate for ACR [144]. For GVHD, the minimum diagnostic threshold is controversial and depends on the apoptotic body count (ABC) observed [145]. The specific number of ABs required for a diagnosis of GVHD may vary. The assessment of crypt apoptosis and ABC is an essential component in the diagnosis of GVHD and ACR in ITx. It is important for pathologists to carefully evaluate and consider the presence and quantity of ABs to make accurate diagnostic interpretations. Furthermore, some studies have found that ABs derived from T lymphocytes are phagocytosed in histological biopsies of patients who have undergone intestinal transplantation [146]. This phenomenon can serve as a basis for early diagnosis of ACR, providing patients with timely diagnosis and treatment opportunities while minimizing intestinal mucosal damage [146].In recent studies, it was found that the number of ABs in patients with oral squamous cell carcinoma (OSCC) without regional lymph node metastasis was significantly higher than that in patients with regional lymph node metastasis, suggesting that the number of ABs may be used as a good parameter to show the possibility of regional lymph node involvement in OSCC patients without lymph node involvement [147]. These findings emphasize the potential utility of ABs as valuable diagnostic biomarkers for assessing cellular rejection and related diseases. By analyzing ABs in biopsy samples, clinicians can gain insights into the underlying pathological processes and make informed decisions regarding patient management and treatment strategies.

ABs as a potential diagnostic biomarker for autoimmune hepatitis (AIH) and embryonal rhabdomyosarcoma (ERMS)

ABs show promise in the diagnosis of AIH. In a study, it was found that the count of ABs in the portal area is associated with the pattern and timing of AIH onset, and it can be used as a diagnostic criterion for AIH [148]. The research revealed that patients undergoing their first liver biopsy had a higher count of ABs compared to follow-up patients, and this count was closely correlated with inflammation. Additionally, incorporating the count of ABs in the portal area as a variable in the AIH scoring system increased the number of patients classified as "definite" AIH [148]. In addition, ABs serve as important indicators for the diagnosis of ERMS [149]. A study conducted on adult females analyzed 25 cases of ERMS and observed that tumor samples exhibited areas of high cell density, often accompanied by ABs and mitotic figures. This suggests a potential role for ABs in the development

Table 4 ABs for disease treatment

Classification	Disease	Animal model	Features	Function of ABs	Refs.
Bone diseases	1. Deossification 2. Hyperosteogeny 3. Osteoporosis 4. OA	1. Murine bone defect model 2. Calvarial defect mice model 3. Parabiosis mouse model 4. Mouse OA model	1. Imbalance between osteogenesis and osteoclast 2. Inflammatory reaction	1. Regulate bone formation/absorption 2. Involve in vascular regeneration and repair 3. Promote bone defect healing 4. Regulate inflammatory factors	[7, 9, 10, 14, 19, 48, 151]
Skin wounds	Back skin wound	Skin wound healing model	Incomplete skin, tissue, inflammatory reaction, and vascular injury	Regulate inflammatory factors, involve in vascular regeneration, and accelerate wound healing	[15, 39]
Tumors	Pancreatic tumors and glioma	Pancreatic tumor microenvironment model and in situ glioma mouse model	Abnormal cell proliferation	Drug delivery, assess drug sensitivity/toxicity, and clear tumor cells	[72, 114, 129, 159, 163]
Immune-related diseases	1. Cutaneous leishmaniasis 2. *Toxoplasma gondii* infection 3. Cancers	1. 4T1 tumor model 2. Murine model of induced immune tolerance	1. Immune tolerance 2. Immunodeficiency 3. Inflammatory reaction	1. Activate the immune system 2. Inhibit autoimmunity 3. Regulate inflammatory factors	[13, 130–132, 165–167, 169, 173]
CVD	MI, Atherosclerosis, and Dox-induced cardiomyopathy	Mouse models of atherosclerosis and MI	Inflammatory reaction, myocardial damage, angiemphraxis, and angiostenosis	Reduce inflammation, repair damaged myocardium, and improve cardiac systolic function	[8, 23, 38, 69, 70, 133, 171]

OA osteoarthritis, *CVD* Cardiovascular diseases, *MI* Myocardial infarction

of ERMS [149]. The study also found co-expression of desmin and myogenin, as demonstrated by immunohistochemical staining, in the tumor samples. The co-expression of these two proteins is a typical feature of ERMS. Additionally, there was a significant increase in proliferative activity, indicated by Ki-67 expression, within the tumor samples. These findings further support the potential association between ABs and the development and progression of ERMS. Therefore, the presence of ABs, their co-expression with desmin and myogenin, and increased proliferative activity can serve as important indicators in the diagnosis of ERMS [149].

Biomolecules carried by ABs are potential biomarkers for disease diagnosis

ABs, as mentioned earlier, have been identified as potential candidates for disease diagnosis based on clinical investigations and histopathological examinations. However, there still exist some limitations such as low sensitivity and difficulty in detecting ABs in hidden areas. ABs extracted from body fluids such as blood, urine, and intestinal tract also have the potential to be used as disease diagnostics. Many studies have shown that compared with the ABs extracted from healthy individuals, the contents of ABs extracted from patients with diseases are different [139]. Therefore, ABs have the potential to develop into clinical samples for disease diagnosis. In addition, for patients, detecting ABs is not only less invasive and risky but also more sensitive to monitoring disease progression than current clinical diagnostic methods such as surgery or pathological examination [136]. By leveraging different techniques such as sample selection, preparation, detection, and analysis, ABs derived from various tissues can offer valuable information for the diagnosis of diverse diseases.

Application of ABs in disease treatment

Bone diseases

Recent studies have shown that ABs are mainly used in the treatment of bone defects, bone hyperplasia, osteoarthritis (OA), osteoporosis, and so on (Table 4). Osteoclast-derived ABs have been demonstrated to promote osteogenesis and are being explored for the treatment of bone defects [7, 9, 19, 48]. These ABs could enhance bone formation and regeneration at specific anatomical sites within the bone. Angiogenesis is closely related to osteogenesis, and promoting angiogenesis during bone repair is essential for ideal bone regeneration [150]. Studies have shown that preosteoclast-derived ABs play a crucial role in promoting endothelial cell proliferation, differentiation, migration, and angiogenesis [7, 9]. On the other hand, abnormal metabolism of osteocytes can easily lead to bone hyperplasia [48]. Studies have found that ABs derived from osteocytes can be targeted to specific parts of the bone for bone resorption, which is expected to be used for the treatment of bone hyperplasia [48]. By utilizing the unique targeting capabilities of osteocyte-derived ABs, it may be possible to selectively reduce excessive bone growth in conditions such as bone hyperplasia. In addition, OA is caused by local inflammation of the joint, and macrophage-derived ABs reverse the inflammatory response caused by M1 macrophages by triggering the polarization of macrophages to the M2 phenotype, indicating that the presence of ABs can effectively reduce the inflammatory response in OA patients [151]. Osteoporosis is characterized by an imbalance between osteoblast and osteoclast activity [152, 153]. In recent studies, ABs provide an opportunity to regulate this imbalance and have the potential to be used in the treatment of osteoporosis. For example, ABs derived from BMSCs have shown the ability to rescue damaged BMSCs and promote bone and fat tissue formation [14]. Additionally, miR-30a released by osteoclast-derived ABs has been found to inhibit osteogenesis. Blocking the release of AB miR-30a presents a potential avenue for treating osteoporosis [10]. In summary, the exploration of ABs in the context of bone diseases opens up new possibilities for therapeutic interventions.

Skin wounds

The repair of skin wounds is an extremely complex process, including initiating coagulation function, regulating inflammatory response, and accelerating the formation of granulation tissue [154]. According to the previous study, the morphology, size, and biomarker of ABs can be characterized through scanning electron microscopy analysis, C1q staining, and Annexin V staining (Fig. 8A–C) [39]. ABs can promote angiogenesis and wound repair

(See figure on next page.)

Fig. 8 ABs promote wound healing. **A** Representative image of scanning electron microscopy analysis analysis of MSC-ABs. **B** Size distribution of MSC-ABs. **C** Representative images of C1q and Annexin V staining of MSC-ABs. **D** Representative images of the H&E staining of the skin samples. **E** Representative images of the Masson staining of the skin samples. **F** The immunofluorescence images and quantification of the CD206 expression level of macrophages treated with different concentrations of ABs. **G** Schema of transplanted MSCs undergo apoptosis after transplantation in a mouse skin wound model and releasing ABs, converting macrophages towards the M2 phenotype and further enhancing the functions of fibroblasts, together contributing to the cutaneous wound healing process. Adapted with permission from ref. [39], image licensed under http://creativecommons.org/licenses/by/4.0/

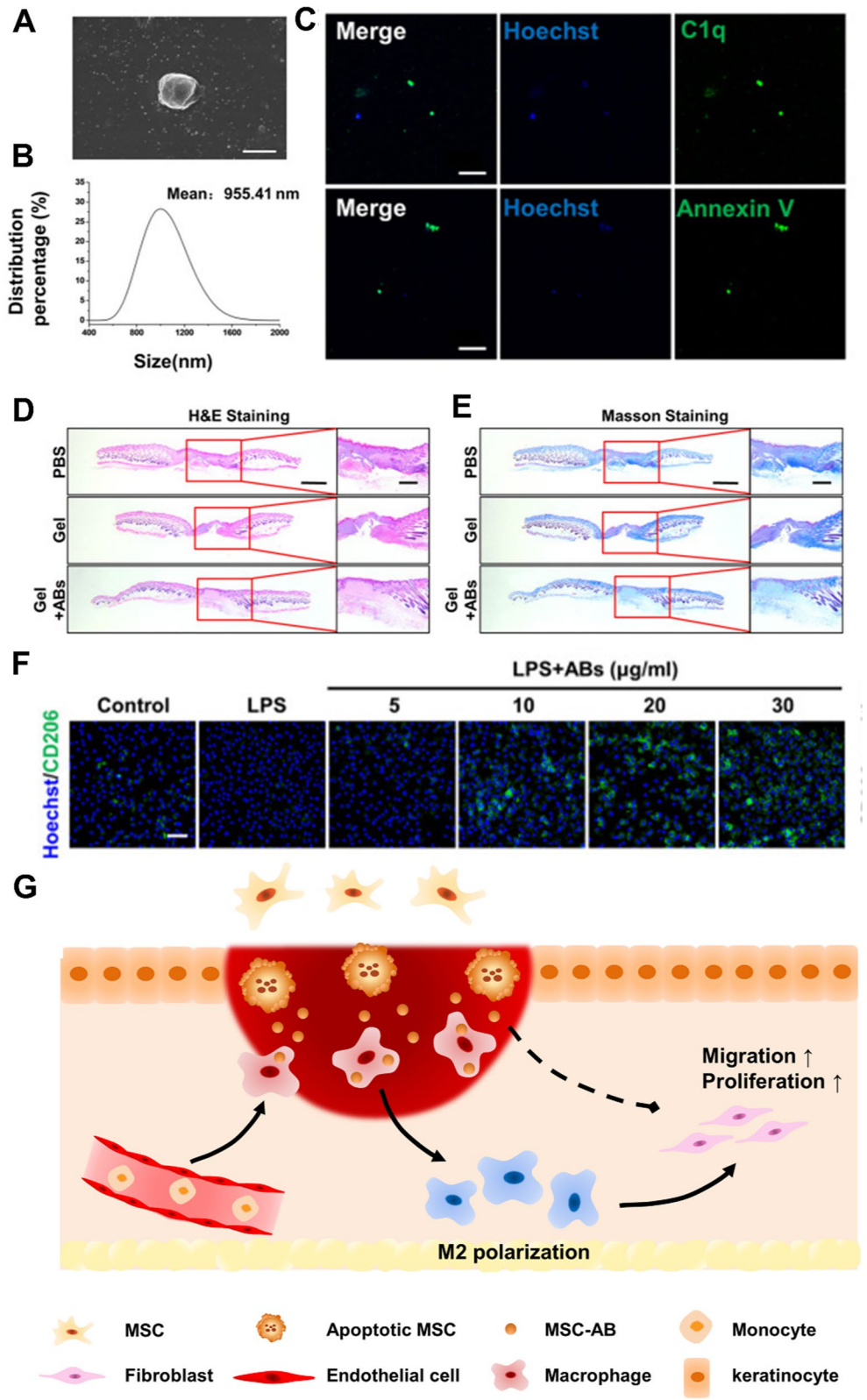

Fig. 8 (See legend on previous page.)

(Fig. 8D, E, G) by inducing macrophage M2 polarization (Fig. 8F) and enhancing fibroblastmigration [15, 39]. At first, researchers found that mesenchymal stem cells can treat skin wounds by anti-inflammatory, promoting angiogenesis, and inhibiting hypertrophic scar formation [155–158]. In a large number of experimental studies, it has been found that mesenchymal stem cells transplanted into animal skin trauma models undergo extensive apoptosis in a short period [39, 155]. To study the effect of apoptosis on the.

therapeutic effect, the researchers compared the therapeutic effects of mesenchymal stem cells and apoptosis-induced mesenchymal stem cells [155]. The results showed that apoptosis-induced mesenchymal stem cells had better inflammatory regulation ability [155]. It is speculated that a large amount of ABs released during apoptosis may be involved in some of the treatment processes [39]. Compared with direct transplantation of apoptotic mesenchymal stem cells, ABs have a higher retention rate and better safety, which is expected to become a better method for the treatment of skin wounds [39].

Tumors

ABs have a good application prospect in the treatment of tumors. In recent years, ABs have been found to enhance drug penetration and predict drug efficacy in tumor treatment [72, 129, 159]. For the treatment of tumors, existing chemotherapy nanomedicines use the proximity effect to increase their penetration into tumors [72]. Proximity effect refers to the observation in vivo that tumor cells ingesting chemotherapeutic drugs release active drugs after apoptosis [72, 160]. By spreading to previously unaffected surrounding tumor cells, apoptosis occurs while surrounding tumor cells ingest the released drugs [72, 160]. Researchers have found that ABs are the largest proportion of EVs produced after the death of tumor cells caused by chemotherapy drugs, so it is reasonable to suspect that ABs can wrap the remaining unused drugs and be swallowed by adjacent tumor cells [72]. By fluorescence staining of the drug, the fluorescence of ABs produced by the drug after acting on tumor cells was observed, indicating that the remaining drug was stored in the ABs released by apoptotic cells [72]. This finding highlights the importance of ABs participating in the proximity effect in the process of drug treatment of tumors and provides new insights into the deep penetration of tumors. On the other hand, many researchers hope to achieve more accurate removal of tumor cells while retaining the ability to deliver drugs by modifying ABs. Studies have shown that tumor-associated macrophages are important participants in the tumor microenvironment [159]. However, there are few anti-tumor drugs targeting these cells in clinical practice [159]. MMP2 is a recognized tumor diagnostic marker [161]. The researchers determined that MMP2-sensitive AB mimic nanoparticles can selectively target tumor-associated macrophages by fluorescence staining experiments [159]. Therefore, it is expected to achieve tumor treatment by loading related anti-tumor drugs on these simulated ABs. In addition, due to the BBB limiting the entry of chemotherapeutic drugs into the brain, non-brain-targeted nanomedicines for brain cancer are not effective [162]. Based on this situation, researchers prepared ABs loaded with doxorubicin and indocyanine green and found that they could actively cross the BBB of glioma mice and release drugs, which significantly prolonged the life span of glioma mice [163].

After cancer patients are treated with chemotherapeutic drugs, the ability to quickly and sensitively predict drug response and toxicity using an in vitro model of patient-derived tumors is crucial for evaluating the efficacy of chemotherapy. At present, the commonly used clinical method is to image the adherent cells after culturing the tumor cells during the treatment process or to detect the fluorescence staining of apoptotic markers by FCM after extracting the tumor cells [129]. The above methods need cell culture and extraction of tumor cells. Researchers have found that drug-treated tumor cells can release ABs in the culture medium, which can be used as potential markers of drug sensitivity without the need to extract cells during culture [129]. However, this method requires the application of high-throughput single-particle impedance FCM to better quantify ABs with compositional diversity [129]. The above research is expected to simplify the process of tumor treatment effect evaluation by further developing related detection techniques and promoting the update and development of clinical detection techniques.

However, ABs may also promote the growth of tumor cells. Studies have found that tumor cell-derived ABs can induce the up-regulation of programmed death ligand 1 in macrophages in the tumor microenvironment [114]. When macrophages express programmed death ligand 1, they can directly bind to programmed death ligand 1 expressed on the surface of tumor cells, thereby inhibiting macrophage phagocytosis of tumor cells [164]. Therefore, further studies are needed to determine the specific role of ABs in the tumor microenvironment if treatment is to be achieved by inhibiting the growth of tumor cells.

Immune disorders

ABs have shown good application prospects in the treatment of immune system-related diseases. Studies have shown that the clearance of ABs in the immune response plays an important role in the maintenance of immune tolerance [165, 166]. For example, patients with systemic lupus erythematosus have the problem of impaired clearance of ABs, which in turn leads to the accumulation of cellular substances containing autoantigens and triggers an autoimmune response [165, 166]. Therefore, researchers are committed to the treatment of autoimmune diseases by studying related substances that can promote the phagocytosis of ABs. Studies have shown that leptin can effectively enhance the phagocytosis of ABs by macrophages in lupus mice [165].

In recent years, researchers hope to use ABs to produce vaccines with more excellent characteristics such as simple manufacture and low toxicity, to achieve the treatment and prevention of related diseases. ABs are selected based on the following characteristics: (1) ABs contain PS and phosphatidylcholine on the surface, which is conducive to the recognition and removal of ABs by antigen-presenting cells such as macrophages [130, 131, 167–169] and (2) ABs contain complex components from dead cells. As a vaccine, ABs can also be used as an immune adjuvant to stimulate specific cellular immune responses and enhance the body's immune response [13, 130, 131, 167, 169]. Researchers induced apoptosis of macrophages infected with Leishmania and Hela cells infected with *Toxoplasma gondii*, and extracted ABs released by apoptotic cells [130, 167]. Subsequent experiments such as lymphocyte proliferation proved that these ABs can stimulate the body to produce an immune response and have a protective effect when attacked by parasites [130, 167].

Clinical trials have shown that dendritic cells that internalize chronic lymphocytic leukemia cell ABs can be used as a vaccine to stimulate the body to produce anti-leukemia T-cell responses [131]. However, due to the limited number of participants in the trial, it is difficult to accurately predict the efficacy [131]. The lack of clinical data highlights the need to develop more effective vaccine strategies in malignant tumors, and it is expected to establish more accurate criteria for evaluating the immune response induced by leukemia vaccines by increasing the number of trials.

CVD

ABs have good application prospects in the treatment of various CVDs, including atherosclerosis, MI, cardiomyopathy, and so on. Atherosclerosis is a long-term chronic disease in which lipids and immune cells are deposited in the arteries, and the plaques formed by deposition are prone to inflammation and damage the vascular structure to affect the blood supply of patients [170]. If no reasonable intervention and treatment measures are taken, coronary artery blockage may occur and lead to MI [170]. Therefore, controlling plaque inflammation is essential for the treatment of atherosclerosis and MI. However, the existing oral or intravenous anti-inflammatory drugs make it difficult to achieve the desired anti-inflammatory effect because they cannot accurately accumulate at the plaque [133]. It can be seen from the previous article that macrophages can recognize and engulf ABs, and ABs can trigger macrophages to polarize to the M2 phenotype to promote anti-inflammatory effects [15, 39]. Therefore, researchers have encapsulated anti-inflammatory drugs into ABs (Fig. 9A) and found that modified ABs can increase the number of anti-inflammatory macrophages (Fig. 9B), which could be efficient for MI and atherosclerosis treatment (Fig. 9C) [38, 133]. This indicates that the presence of ABs can enable anti-inflammatory drugs to accurately target plaques and enhance the body's anti-inflammatory response, highlighting the potential of ABs as a therapeutic measure for atherosclerosis. On the other hand, repairing vascular injury is also one of the important therapeutic approaches. Studies have shown that ABs derived from ECs promote the repair of vascular endothelium and reduce atherosclerotic plaques [8, 23, 171].

Cardiomyopathy is a group of myocardial organic diseases caused by multiple causes. At present, there is no specific treatment for the disease in the clinic [172]. The treatment principle is to improve the clinical symptoms and improve the long-term survival rate [172]. However, studies have shown that stem cells can repair damaged myocardium, a method that may achieve a cure for cardiomyopathy [69, 70, 171]. Researchers have found that ABs of cardiomyocytes and fibroblasts regulate the directional differentiation of CSCs [69, 70, 171]. Among them, cardiomyocyte-derived ABs increase myocardial contractility, while fibroblast-derived ABs decrease myocardial contractility [69, 70, 171]. It is necessary to further study the specific mechanism of ABs acting on the myocardium in order to develop targeted drugs that can affect the differentiation direction of stem cells in myocardium and other organs.

Other diseases

The pathogenesis of type 2 diabetes mellitus is diverse. Among them, excessive infiltration of macrophages and pro-inflammatory reactions can cause relatively insufficient insulin secretion resulting in elevated blood glucose, which eventually leads to the occurrence of type 2 diabetes mellitus [174]. Studies have shown that

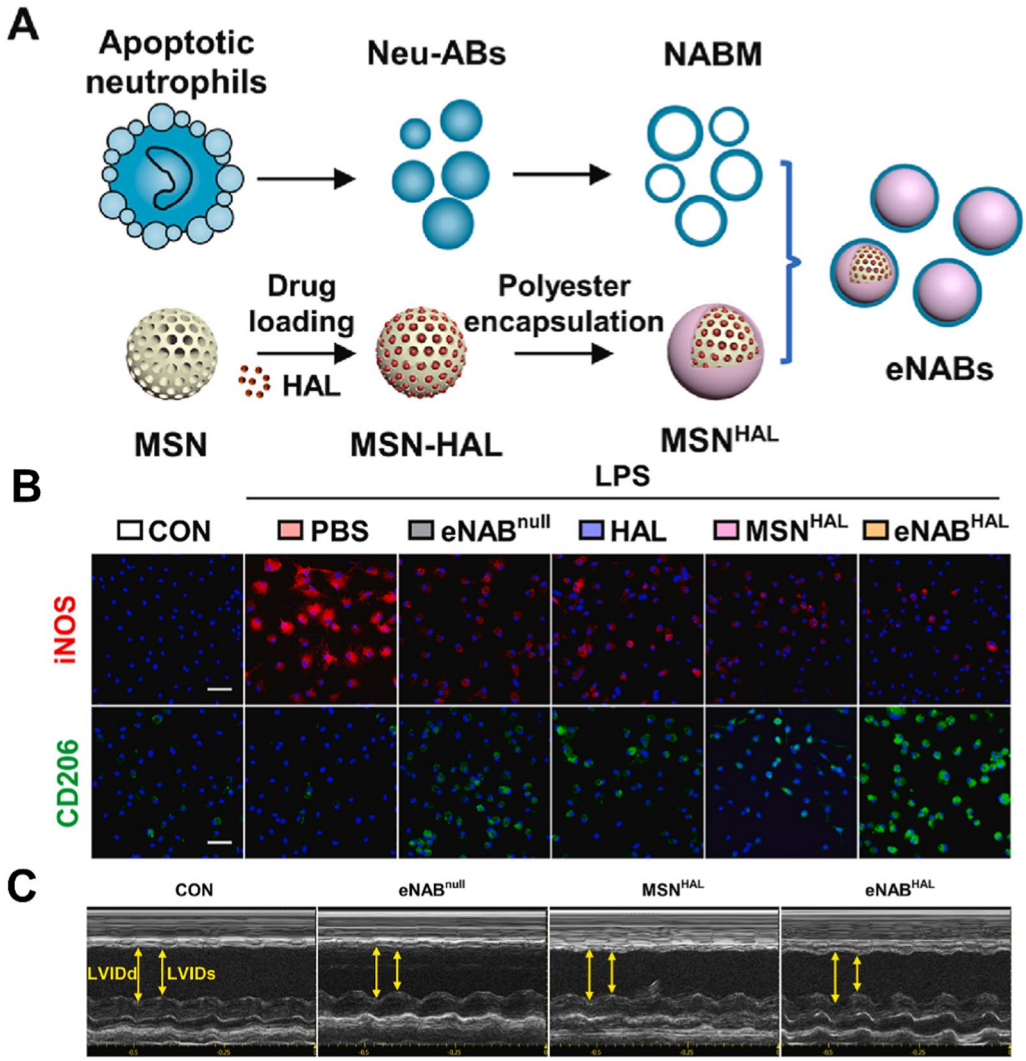

Fig. 9 Engineering ABs for therapeutic application. **A** Schematic of engineered neutrophil ABs (eNABs) for MI treatment. **B** Representative fluorescence images of the macrophage phenotypes and the percentage of the iNOS/CD206-positive population. **C** Representative echocardiographic images for various groups after 4 weeks. Distance between yellow arrows indicates left ventricular internal end-diastolic dimension (LVIDd) and left ventricular internal end-systolic dimension (LVIDs), respectively. Adapted with permission from ref [38]

ABs derived from BMSCs induce the reprogramming of macrophages at the transcriptional level, thereby inhibiting the aggregation of macrophages in the liver and promoting the transformation of macrophages into anti-inflammatory phenotypes [40]. The endoplasmic reticulum (ER) calcium-binding protein (CRT) is exposed to the surface of ABs, which mediates the uptake of ABs by macrophages, and reduces macrophage infiltration and pro-inflammatory response, thereby improving the body's glucose tolerance, reducing insulin resistance, and improving liver steatosis [40]. These findings highlight the ability of ABs to regulate the homeostasis of liver macrophages, but the response of CRT to other cells is still uncertain. Therefore, it is necessary to further study the signal molecules and pathways involved in the treatment of type II diabetes by ABs.

Intrauterine adhesions are caused by uterine trauma leading to endometrial fibrosis and partial or total occlusion of the uterine cavity [175]. Studies have shown that ABs derived from mesenchymal stem cells reduce endometrial fibrosis and induce endometrial regeneration by inducing macrophages to transform into anti-inflammatory phenotypes, promoting cell proliferation and angiogenesis [134]. The results showed that the injection of ABs-loaded hyaluronic acid hydrogel could effectively repair the endometrium and restore the

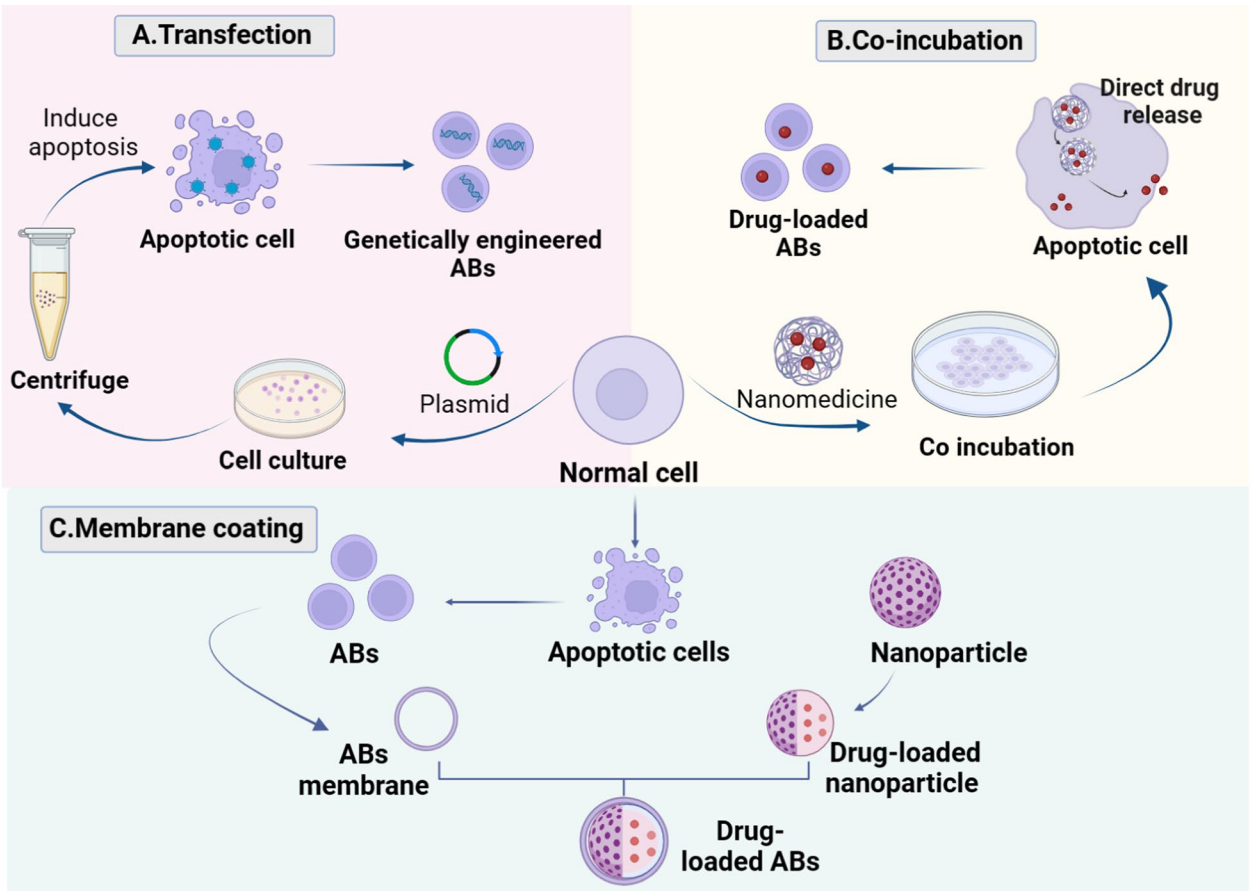

Fig. 10 Drug loading strategies for ABs. **A** transfection, **B** co-incubation, and **C** membrane coating. Created with BioRender.com

fertility of the body [134]. The above studies have shown the good potential of ABs as therapeutic vesicles, which is expected to develop a clinically feasible alternative method for the treatment of intrauterine adhesions.

Tailoring of ABs

ABs, as special EVs, not only have potential in disease diagnosis but also show excellent therapeutic potential by delivering various biomolecules for intercellular communication. ABs have been designed in various ways for disease treatment, especially in drug delivery. In recent years, a series of drug-loading strategies based on ABs have been developed to deliver active drug components to various target organs, tissues, or cells [74, 176]. In order to further enhance the therapeutic effect of ABs, a series of engineering modification methods have been developed such as surface modification of ABs and biomimetic ABs [177]. The recent progress emphasizes the potential of engineered ABs for drug delivery. In this section, we describe the summarized drug-loading strategies of ABs as well as engineering modification methods and biomimetic strategies.

Construction of engineered drug-loaded ABs

At present, drug loading of ABs can be achieved by transfection, co-incubation, and membrane coating (Fig. 10). Transfection refers to the introduction of exogenous RNA or DNA fragments into cells under certain specific conditions to obtain new phenotypes and functions [178]. To obtain ABs cell proliferative ABs, miR-221 can be transfected into macrophage-derived ABs using Exo-Fect reagent [67]. Furthermore, to enable drugs to treat diseases through the BBB, a variety of methods have been developed to break this barrier. Among them, the use of ABs membranes to mediate drug brain delivery is very important. The anti-inflammatory drug antisense oligonucleotides were combined with cationic konjac glucomannan (cKGM) to form a CKA complex, which was transfected into cells through the combination of cKGM and the mannose receptor on the cell surface. Ultraviolet irradiation-induced ABs exert anti-inflammatory effects. CD44v6 on the surface of ABs facilitates the penetration of the blood–brain barrier and achieves drug delivery in the brain [176]. In addition, to obtain ABs that can

enhance the immune response, influenza virus hemagglutinin (HA) and nucleoprotein (NP) were introduced into cells through transient transfection, and these ABs can be engulfed by antigen-presenting cells (APC) to enhance the immune response of T and B cells, providing new ideas for immunotherapy [179].

In addition to cell transfection, co-incubation is a commonly employed method for loading drugs. During co-incubation, drugs are exposed to cells, and in some cases, certain drugs can directly induce apoptosis, resulting in the formation of drug-loaded ABs. For example, research has shown that loading cisplatin and the hypoxia-activated prodrug PR104A into nanoparticles and co-cultivating with 4T1 cells can directly kill externally nonmonic tumor cells with cisplatin and form ABs, while PR104A enters the hypoxic tumor cells within ABs to further exert its cytotoxic effects, achieving deep penetration in tumors [180]. However, for some drugs, artificial induction of apoptosis is required within the cells to obtain ABs loaded with the drugs. For instance, another approach is to co-cultivate cytosine-phosphoric acid-guanine (CpG) adjuvant with 4T1 cells and induce apoptosis with streptolysin, resulting in ABs carrying CpG as cancer vaccines. This vaccine can target macrophages and promote their polarization into M1 type, improving the immune-suppressive microenvironment and enhancing cancer treatment efficacy [132]. Moreover, CpG can also be modified onto gold and silver nanorods, which are then co-incubated with tumor cells to generate ABs loaded with nanodrugs. Based on the photothermal effect of nanorods and the immune activation ability of CpG, these ABs can not only effectively eliminate primary tumors but also prevent tumor metastasis and recurrence [181]. In addition to CpG, Cyclic 2′,3′-GMP-AMP (cGAMP), known as a stimulator of interferon genes (STING) pathway agonist, has emerged as a promising immunotherapy drug capable of enhancing innate immunity [182–184]. When cGAMP is co-incubated with tumor cells, the generated ABs are easily engulfed by APCs, thereby augmenting the body's immune response [185].

The membrane of ABs contains various 'eat me' signaling molecules, such as PS, ICAM-3, C1q, and others, which enable specific uptake of ABs by cells [159, 186]. Utilizing ABs in drug delivery processes can enhance delivery efficiency. However, the large content of ABs significantly reduces the drug load, necessitating further investigation into its safety. Therefore, separating the ABs membrane for drug encapsulation not only improves the loading efficiency but also increases the drug stability. Dou et al. isolated ABs membrane from T cells by ultrasonic treatment to form apoptotic ghosts, which were pre-loaded with Mesoporous Silica Nanoparticles (MSNs) containing anti-inflammatory agents miR-21 and curcumin [187, 188]. Purified AB ghosts were wrapped on the surface of MSN, forming chimeric ABs (cABs) [128]. Due to the inflammatory targeting and drug release properties of AB ghosts, cABs can target macrophages in inflammatory regions and promote their polarization into M2 type, regulating inflammatory response [128]. Another method involves obtaining neutrophil-derived ABs membrane (NABM) through ultrasonic treatment and mixing with MSNs carrying the anti-inflammatory drug hexyl 5-aminolevulinate hydrochloride (HAL), followed by co-extruded to engineered neutrophil ABs (eNABs) that actively target macrophages [38]. After drug release, eNABs produce anti-inflammatory bilirubin, further enhancing the anti-inflammatory effect [38]. Furthermore, copper-modified nanoparticles with antioxidant properties can be encapsulated in NABM to improve local inflammatory response [189].

Surface functionalization of ABs

As a type of EVs, ABs possess an outer membrane that comes from the cell membrane, exhibiting the structure of a phospholipid bilayer, protein, and cholesterol. According to the characteristics of membrane structure, numerous modification methods have been developed in recent years to enhance the functionalities of these EVs, including therapeutic capabilities, targeted delivery, evasion of clearance mechanisms, and more [190–194]. Specifically, several strategies have been employed, including pre-isolation modification and post-isolation modification (Fig. 11).

Pre-isolation modification

Pre-isolation modification includes genetic, metabolic, and direct parent cell membrane engineering approaches. Among them, genetic engineering refers to the enrichment of target proteins in EVs by transfecting specific gene fragments into parental cells. Surface-modified EVs by genetic engineering are endowed with targeting and specific [195–198], and can also be used in engineered EVs to allow fluorescent, luminescent, or radioactive tracking [199, 200]. Metabolic engineering produces EVs with modified proteins, lipids, or glycans on the surface by adding synthetic modified amino acids, lipids, glycans, or oligonucleotides to the culture medium of parental cells. Through the endogenous synthesis and modification process of cells, the need for gene manipulation is avoided and the functionalization of EVs is achieved [201–204]. Direct parent cell membrane engineering directly transforms the parental cell membrane. By fusing functional liposomes with the parental cell membrane, EVs containing a special membrane surface

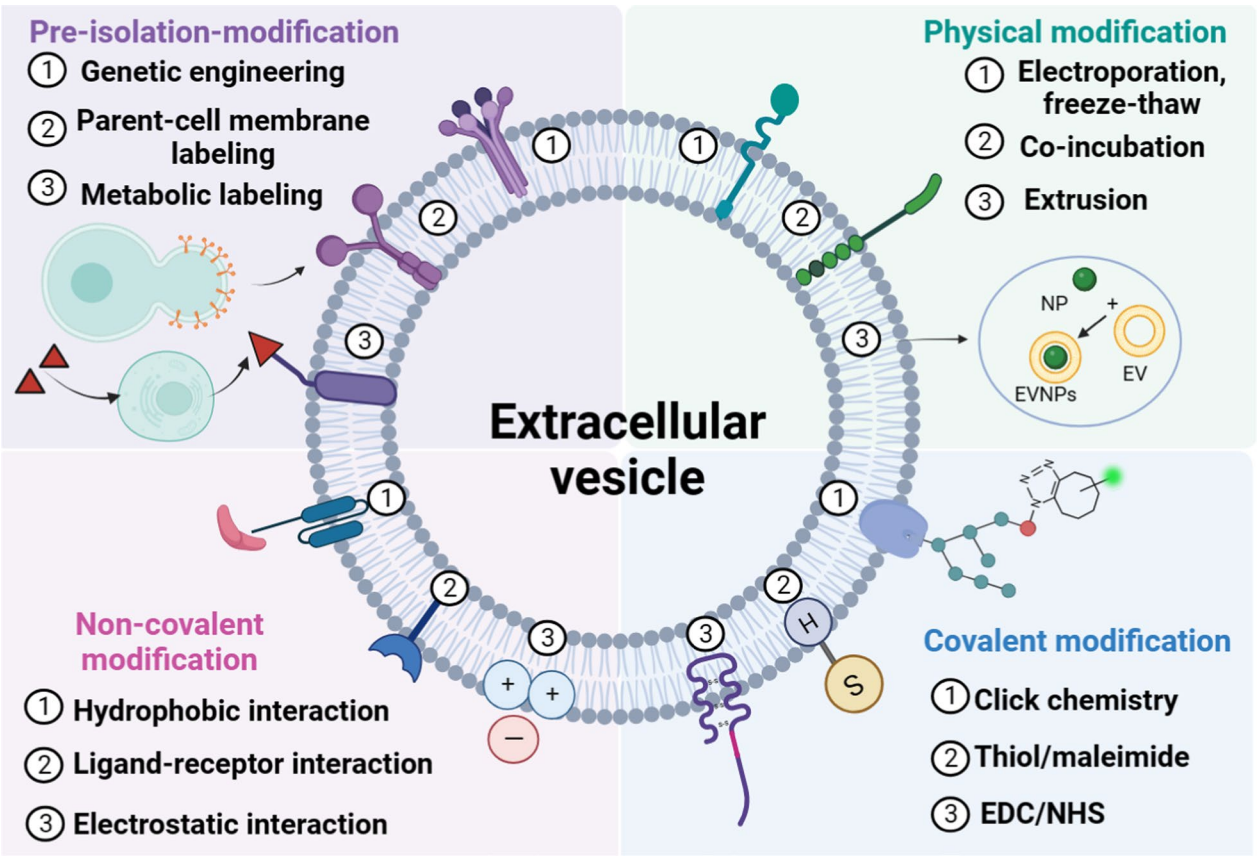

Fig. 11 Potential feasible methods for surface modification of ABs. Created with BioRender.com

are obtained. This approach applies to engineering EVs with various functional moieties[205].

Post-isolation-modification

Post-isolation modification can be roughly divided into methods based on physical interactions and methods based on chemical modifications on the vesicles' surface. At present, the common physical surface engineering methods of EVs include electroporation, freeze–thaw, extrusion, and co-incubation. These methods can temporarily destroy the lipid structure of the membrane and allow the EV membrane to penetrate for cargo loading and surface functionalization. Electroporation is a technology that uses an electric field to temporarily destroy the membrane structure, which allows exogenous substances to enter the membrane of EVs, thereby improving the targeting ability of EVs [206–208]. In addition to electroporation, freeze–thaw is also an important method for surface penetration and modification of EVs. Using freeze–thaw technology, the peptide and EVs are shaken together in an ice bath to integrate the target peptide onto the surface of EVs [209–211]. Extrusion is a common physical modification technique that encapsulates EV films onto different nanoparticles, which are protected from phagocytosis by cells by encapsulating nanoparticles [212–215]. Co-incubation is a common method to introduce targeted peptides into the surface of EVs. This process involves incubating EVs with a targeted peptide, and EVs containing the targeted peptide on the surface can bind to specific receptors on the target cells [216].

Chemical modification

Chemical modification includes covalent modification and non-covalent modification. The widely used strategies for covalent conjugation include the biorthogonal click chemistry, the thiol–maleimide coupling, and EDC–NHS coupling [217–219]. Click chemistry azide–alkyne cycloaddition is a reaction between an alkyne and an azide, involving the catalysis of a triazole linkage by copper. The thiol–maleimide coupling reaction involves adding maleimide to the sulfhydryl groups on the surface of EVs. EDC–NHS coupling could be used for conjugating peptides, proteins, antibodies, and so on to the AB surface. Unlike covalent modifications, non-covalent modifications correspond to AB conjugation

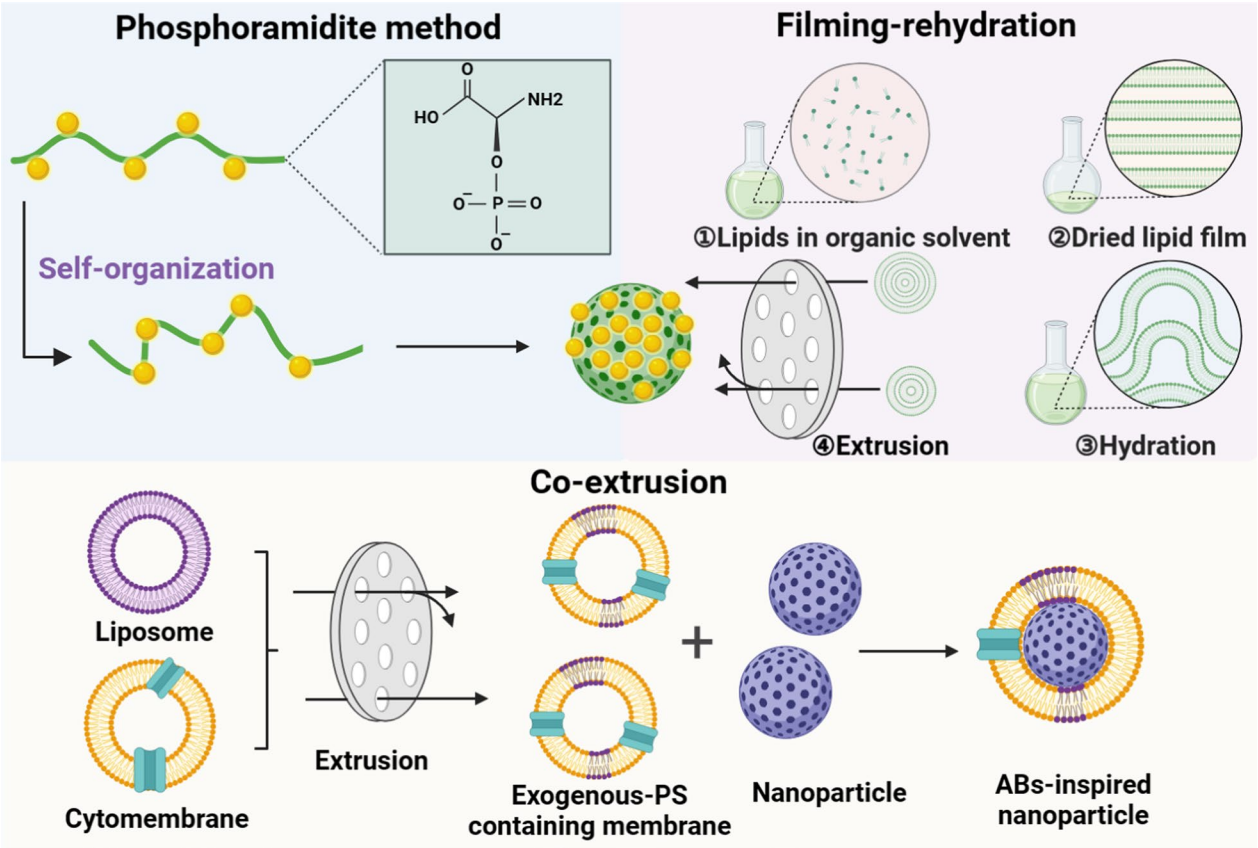

Fig. 12 Engineering modification of ABs. Created with BioRender.com

via weak interactions, including electrostatic, hydrophobic, or ligand-receptor interactions by nature [203, 220–222]. The surface modification of ABs by electrostatic interaction is achieved by adding functional fragments, which can make ABs positively charged and target negatively charged target cells, and enhance the targeting of ABs to biofilms. The hydrophobic interaction between the vesicles and the liposomes, the liposome membrane was functionalized with peptides, antibodies, or polyethylene glycol (PEG), and then the outer vesicles and the liposome membrane was fused by freeze–thaw method to improve the targeting of the outer vesicles. Ligand-receptor interactions take advantage of the natural receptors present over the extracellular surface vesicles to attach ligands to improve ABs' target specificity by ligand coupling [216, 223, 224]. In a word, the surface modification methods for ABs include pre-isolation-modification i.e., genetic, metabolic, and direct parent cell membrane engineering methods, and post-isolation-modification i.e., physical interactions and chemical modifications [225–228]. Although these methods can improve the function of EVs, there are still many problems to be solved before these functionalized external vesicles, including ABs, can be applied to the clinic, such as safety issues and clinical evaluation [229–231].

Biomimetic ABs

Natural ABs are limited in terms of stability and yield, making it challenging to utilize them for large-scale clinical therapy. To address this issue, a series of synthetic "biomimetic ABs" strategies have been developed. These strategies involve simulating the “eat me” signal of ABs to prepare nanomaterials or liposomes that contain PS (Fig. 12) [232]. Nanomaterials are extensively employed in biomedical research due to their distinctive physical and chemical properties. Drawing upon the ease of recognition and engulfment exhibited by ABs, researchers have devised a strategy to develop ABs-triggered nanoparticles. According to this strategy, PLGA nanoparticles were first synthesized, and then ABs-inspired nanoparticles were obtained by co-extrusion by coating cell membrane containing PS on PLGA nanoparticles [232–240]. These AB-inspired PS/membrane-coated nanoparticles (PS-MNPs) are better engulfed by macrophages and have anti-inflammatory properties. To

enhance the performance of the nanomaterial, researchers further modified it by incorporating acid-sensitive PEG. This modification enables the nanomaterial to not only exhibit the phagocytic properties of ABs and anti-inflammatory effects but also respond to inflammation through pH changes [233]. In addition to modifying PS on the surface, researchers have constructed an apoptotic cell-inspired deformable PS-containing nanoliposome. Compared with the nanomaterials simply modified with PS, the elasticity and surface properties of the coupled nanomaterials can better regulate the macrophage-mediated inflammatory response, and bind to macrophages for a longer time and better effect [235, 236]. The softness of nanomaterials may enhance the therapeutic effect of macrophage-mediated nanosystems, which will provide new ideas for the design of engineered nanotherapeutic drugs.

In addition to the extrusion methods, AB-inspired materials can also be obtained by the filming-rehydration method. In this method, the lipid film was first prepared, and then a suitable aqueous medium was added. After that, the liposome was mixed with PS, homogenized by an ultrasonic probe, and obtained by a microporous membrane filter [133, 241–243]. In the preparation process of PS liposomes, drugs such as metformin and alendronate can also be added, and the drugs can be transported to the target position by the targeting effect of PS [236, 241, 242].

In addition to filming-rehydration and co-extrusion, biomimetic ABs can also be achieved by using apoptotic cell membrane-inspired phosphoserine polymers. In 2017, researchers demonstrated for the first time that phosphoserine polymers inspired by apoptotic cell membranes can protect mouse macrophages from lipopolysaccharide-induced inflammation and inhibit macrophage activation. In this study, the researchers synthesized water-soluble methacryloyloxyethyl phosphorylserine (MPS) using the phosphoramidite method. Firstly, they used 2-hydroxyethyl methacrylate and tert-butyl tetraisopropyl phosphate diamine as raw materials to synthesize MPS by the phosphoric acid diamine method. Secondly, they synthesized MPS and phosphorylcholine at a copolymerization ratio of 1: 9, which mimics apoptotic cell membranes [244]. In summary, the biomimetic ABs strategy has led to the development of various biomimetic materials through extrusion, filming-rehydration, phosphoramidite method, and other methods. It is anticipated that more nanomaterials will be developed in the future utilizing ABs for the treatment of various diseases.

Challenges and prospects

As cell-derived natural vesicles, ABs have biological advantages over other nanomaterials [245]. In addition, they carry effective biological molecules, which makes them ideal candidates for disease diagnosis and treatment [128–136]. In recent years, a series of engineered modification strategies have been developed to expand the therapeutic targeting and efficacy of ABs, to maximize the therapeutic effects [190–194]. The strategies include drug-loading ABs, surface modification strategies, and biomimetic ABs. Owing to numerous modification methods, ABs have been utilized in various medical fields, including but not limited to drug delivery, tissue regeneration, immunotherapy, vaccine development, and disease therapy. Although engineered ABs possess advantages compared with natural ABs, the clinical translation of engineered ABs faces many challenges. First, although multiple methods for ABs isolation and characterization exist, there is still a lack of standardization in efficient separation and identification strategies, which hinders the large-scale production of ABs for clinical use. Furthermore, the loading efficiency of therapeutically effective medications remains insufficient, which makes its improvement a primary focus of future research. Lastly, the surface modification of ABs could lead to the disruption of ABs membrane structure, thus affecting the structure stability, cellular uptake pathways, and regulatory function of ABs. In conclusion, despite some potential setbacks and challenges, engineering-modified ABs hold promise for robust use in clinical settings, and further studies are needed to assess the safety and efficacy of the new generation of engineered ABs.

Conclusion

In summary, the biological characteristics and physiological functions of ABs make them promising therapeutic agents. It has been found that engineering manipulation of ABs can enhance their functions, including therapeutic potential, targeted delivery, evasion of clearance mechanisms, and more, thus accelerating the progress of the clinical application of ABs. However, given the shortcomings of the current modification methods of ABs, more strategies are needed to solve the safety and functional integrity issues, which may make the clinical application of ABs more feasible and provide new prospects for the diagnosis and treatment of various diseases.

Abbreviations

ABC	Apoptotic body count
ABs	Apoptotic bodies
ACR	Acute cellular rejection
AIH	Autoimmune hepatitis

AKT	Protein kinase B
Ambra1	Activating molecule in beclin1-regulated autophagy
AMN	Apoptotic microtubule network
APC	Abdominal antigen-presenting cells
Ara-C	Cytosine arabinoside
ATF6	Activating transcription factor 6
BAI1	Brain-specific angiogenesis inhibitor 1
BBB	Blood–brain barrier
BMM	Bone marrow macrophage
BMSCs	Bone marrow mesenchymal stem cells
cABs	Chimeric ABs
Caspase-3	Cysteine protease 3
CDKN1B	Cyclin-dependent kinase inhibitor 1B
cGAMP	Cyclic 2′,3′-GMP-AMP
cKGM	Cationic konjac glucomannan
COX2	Cyclooxygenase-2
CpG	Cytosine-phosphoric acid-guanine
CRT	Calreticulin
Cryo-TEM	Low-temperature transmission electron microscopy
CSCs	Cardiac stem cells
CSF	Cerebrospinal fluid
CVD	Cardiovascular diseases
CYTC	Cytochrome C
ECs	Endothelial cells
eNABs	Engineered neutrophil ABs
EP	Prostaglandin receptor
EPCs	Endothelial progenitor cells
ER	Endoplasmic reticulum
ERMS	Embryonal rhabdomyosarcoma
EVs	Extracellular vesicles
FACS	Fluorescence-activated cell sorting
FCM	Flow cytometry
GlcNAc	N-acetylglucosamine
GLPMs	GATA6 large peritoneal macrophages
GVHD	Graft-versus-host disease
HA	Hemagglutinin
HAL	Hydrochloride
HGF	Horizontal gene transfer
HIF-1α	Hypoxia-inducible factor-1α
HUVACs	Human umbilical vein endothelial cells
ICAM	Intercellular adhesion molecules
ICG	Indocyanine green
IFN	Interferon
IGF2BP	3 Insulin growth factor 2 mRNA-binding protein 3
IL	Interleukin
iPGE2	Intracellular prostaglandin E2
IRE1	Inositol-requiring transmembrane kinase endoribonuclease-1
ITx	Intestinal transplants
LFA-1	Lymphocyte function-associated antigen 1
LIMK1	Lim domain kinase 1
lncRNAs	Long non-coding RNAs
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MerTK	Tyrosine-protein kinase Mer
MI	Myocardial infarction
miRNAs	MicroRNAs
MLCK	Myosin light chain kinase
mOC	Mature osteoclast cell
MPS	Methacryloyloxyethyl phosphorylserine
MSC	Marrow mesenchymal stem cells
MSNs	Mesoporous Silica Nanoparticles
mTOR	Mammalian target of rapamycin
NABM	Neutrophil-derived ABs membrane
NO	Nitric oxide
NP:	Nucleoprotein
NTA	Nanoparticle tracking technology
OA	Osteoarthritis
OSCC	Oral squamous cell carcinoma
PAK2	P21-activated kinase
PANX1	Pan-catenin 1
PDGF-BB	Platelet-derived growth factor-BB
PD-L1	Programmed cell death ligand 1
PERK	Protein kinase RNA–like endoplasmic reticulum kinase
PI3K	Phosphoinositide 3-kinase
PlexB2	Plexus B2
pOC	Pre-osteoclast cell
poly (MPC)	Synthesized MPS and phosphorylcholine
PS	Phosphatidylserine
PS-MNPs	PS/membrane-coated nanoparticles
PTPRC	Protein tyrosine phosphatase receptor C
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa B ligand
RCCS	Rotating cell culture system
RGS	Regulator of G protein signaling
ROCK1	Rho-associated protein kinase 1
S6K	S6 kinase
SMAD2	Drosophila mothers against decapentaplegic protein 2
STING	Stimulator of interferon genes
TEM	Transmission electron microscopy
TFEB	Transcription factor EB
TGF-β	Transforming growth factor-β
Th1	T helper 1
TIM	T cell immunoglobulin mucin proteins
TNF	Tumor necrosis factor
TSP	Thrombospondin
VEGF	Vascular endothelial growth factor

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Competing interests
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