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# Exploiting Sound for Emerging Applications of Extracellular Vesicles

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## Abstract

Extracellular vesicles are nano- to microscale, membrane-bound particles released by cells into extracellular space, and act as carriers of biomarkers and therapeutics, holding promising potential in translational medicine. However, the challenges remain in handling and detecting extracellular vesicles for disease diagnosis as well as exploring their therapeutic capability for disease treatment. Here, we review the recent engineering and technology advances by leveraging the power of sound waves to address the challenges in diagnostic and therapeutic applications of extracellular vesicles and biomimetic nanovesicles. We first introduce the fundamental principles of sound waves for understanding different acoustic-assisted extracellular vesicle technologies. We discuss the acoustic-assisted diagnostic methods including the purification, manipulation, biosensing, and bioimaging of extracellular vesicles. Then, we summarize the recent advances in acoustically enhanced therapeutics using extracellular vesicles and biomimetic nanovesicles. Finally, we provide perspectives into current challenges and future clinical applications of the promising extracellular vesicles and biomimetic nanovesicles powered by sound.

### Keywords

extracellular vesicles; exosomes; biomimetic nanovesicles; acoustics; disease diagnostics; therapeutics

## 1. Introduction

Extracellular vesicles (EVs) carrying various bioactive molecules including proteins, DNAs, and RNAs, from their source cells, have been used for disease diagnosis and therapeutics.[1–3] EVs are present in biological fluids and play a critical role in multiple

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physiological and pathological processes.[4–6] Serving as cell-cell communicators, EVs can be decoded to obtain developmental information about diseased cells or tissues.[7–9] Furthermore, due to their inherent ability to carry intracellular secretions, EVs can be manipulated and engineered to load drugs and regulate cell functions for targeted therapies. Despite these potential benefits, the wide application of EVs is hampered due to their heterogeneity and rarity.[10–12] To address the challenges, biomimetic nanovesicles, such as cell-derived membranes and protein-bound gas vesicles, have been rapidly developed recently.[13–15] These biomimetic nanovesicles can be manufactured and purified from microbes like cyanobacteria and haloarchaea on a uniform and large scale, making them well-suited for diagnostic applications.[16–19] Moreover, the biomimetic nanovesicles can inherit the biocompatibility and long-term stability of EVs, rendering them a promising drug delivery tool for in vivo disease treatment.[20–23] Importantly, these biomimetic nanovesicles could also be designed specifically via chemical methods to target certain cells, reducing the risk of side effects.[24–26] Although EVs and biomimetic nanovesicles hold tremendous potential in disease diagnosis and therapeutics, the challenges remain in handling, generating, and detecting them as well as exploring their controllability and responsiveness for disease diagnosis and treatment. Thus, there are tremendous unmet needs to develop novel technologies for exploring emerging applications of EVs and biomimetic nanovesicles.[27, 28]

Sound waves seem to be a promising method for addressing the current challenges of EVs and biomimetic nanovesicles and extending their applications in disease diagnosis. So far, sound waves have been widely used for life sciences and clinical medicine.[29–40] As a kind of mechanical vibration, sound waves can provide contactless, label-free, and highly biocompatible manipulation in the spatiotemporal dimension by creating desired acoustic pressure and acoustic streaming fields.[41–47] In particular, the forces and effects derived from sound waves can act directly and remotely on nano to micro-scale bio-objects such as EVs.[48–53] Through the integration with microfluidics and microfabrication,[54–62] the acoustofluidic devices and acoustic microdevices have been developed and utilized to separate, purify, and enrich EVs from patient samples, as well as accelerate the generation of EVs from cells.[63–65] The contents of isolated or generated EVs are analyzed for providing information on the presence of diseases for disease diagnosis or biomarker discovery. Moreover, sound waves can facilitate biosensing and real-time imaging of EVs for disease detection and diagnostic applications through their interaction with EVs and biomimetic nanovesicles.[66–68] These acoustic technologies offer advantages such as rapid isolation, high purity, and minimal damage to the EVs, improving EV-based disease diagnosis and biomarker discovery.

Sound waves have been used to be a promising tool for extending the therapeutic application of EVs and biomimetic nanovesicles. Sound waves can reversibly alter the structure and morphology of nanovesicle membranes, allowing for the engineering and modification of vesicles at the molecular level.[69–71] For instance, sound waves such as ultrasound beams can improve drug loading[72], transportation[73], and release[74] from these vesicles for drug delivery applications. Moreover, sound waves such as low-intensity ultrasound can enhance the generation of EVs by regulating EV biosynthesis, shining light on the large-scale production of EVs, and making them a promising tool for translational medicine

applications. So far, the therapeutic combinations of sound waves and nanovesicles have shown promise in treating various diseases including cancer, neural disorder, etc.[75–84] For example, sound waves can increase the permeability of the blood-brain barrier (BBB), allowing to delivery of large therapeutics into brain regions. Sound waves are non-invasive and can be applied remotely, which makes them a safe and convenient tool for delivering EVs to target tissues. Sound waves can be focused on a specific area of the human body, allowing for targeted delivery of EVs to specific tissues or organs. Sound waves can be used at low intensities, which can reduce damage to the EVs and target tissues. Sound waves can increase the efficiency of EV delivery by increasing their uptake by target cells.[85, 86] Therefore, the combination of sound waves and EVs would significantly improve the therapeutic potential of EVs and biomimetic nanovesicles.

In this review, we summarize recent emerging applications of EVs and biomimetic nanovesicles powdered by sounds from the basic principle to their translational applications. Our objective is to introduce the unique roles and physical mechanisms of sound waves in various theranostic applications of EVs and biomimetic nanovesicles (Fig. 1). Specifically, to improve the current technologies of EVs and biomimetic nanovesicles, we elaborate on the fundamental effects of sound waves, namely, mechanical effect, acoustic cavitation, thermal effect, chemical effect, scattering effect, and piezoelectric effect. Moreover, we summarize the recent advances in employing these effects in the enrichment, manipulation, biosensing, bioimaging, and excitation of EVs and biomimetic nanovesicles for translational medicine. We conclude by offering perspectives on current challenges and future clinical applications of EVs and biomimetic nanovesicles powered by sound.

## 2. Sound waves acting on EVs

Sound is a form of mechanical energy, and can handle and manipulate micro- to nano-scale objects using various physical principles.[40, 87–92] The fundamental physical mechanisms behind the acoustic manipulation of EVs may involve mechanical effects, cavitation, thermal effect, chemical effect, scattering effects, piezoelectric effect, and their combinations (Fig. 1). The mechanical effect primarily contributes to the major forces for EV manipulation, such as the acoustic radiation force, and hydrodynamic force driven by acoustic streaming, enabling the handling of EVs in the acoustic pressure field.[93–95] By adjusting the frequency and intensity of the acoustic waves, EVs can be manipulated and moved in the desired direction.[96, 97] Acoustic streaming,[98–101] on the other hand, acts on EVs by generating fluid momentum through acoustic energy attenuation. In addition to the mechanical effect, acoustic cavitation is another common mechanism explored in biological applications, where tiny bubbles are formed, grown, and even collapsed due to the pressure distribution.[102–104] These oscillating bubbles generate stable microstreaming within biofluids, altering the membrane’s permeability and generating strong mechanical pressure.[105, 106] Sound waves also can generate cycles of compression and expansion in different regions, creating significant heat (thermal effect).[107, 108] The compression cycles exert a positive pressure on the fluid, pushing molecules together, while the expansion cycles exert a negative pressure, pulling molecules apart. This creates an unusual environment with vigorous molecule motions that can facilitate sonochemical reactions, mainly relying on the quick heating and cooling caused by acoustic cavity implosion.[109–112] Scattering

effect occurs when sound waves hit a structure with different acoustic impedance to the surrounding tissue that is smaller than the wavelength of the incident sound wave.[113–115] The constructive and destructive interference of the scattered waves produces different intensities of echoes, enabling the visualization of various bio-objects. Conversely, EVs and nanovesicles can also affect the sound, with the piezoelectric substrate precisely sensing their contact by reading the electric signal derived from the change in vibration (piezoelectric effect).[116, 117] Therefore, the interactions between sound and EVs and nanovesicles can be categorized in the principles described above.

## 3. Diagnostics

EVs are promising biomarkers for disease diagnostics because they contain various cell-derived signaling molecules, DNAs, RNAs, proteins, and lipids that can reflect abnormalities of organisms at the cellular level.[118–124] Attractively, sound waves can be a valuable tool in exploring and improving the biosensing and bioimaging of nanoscale exosomes and biomimetic vesicles, facilitating the more effective evaluation of gene expression, protein biosynthesis, cell function, and tissue development.[125, 126] In this section, we review the diagnostic values of EVs leveraged by sound waves, based on their ultrasonic responsiveness, genetic information, functional heterogeneity, and scattering properties.[127]

### 3.1 Handling of extracellular vesicles.

Given the tremendous potential of EVs for diagnostic purposes, extensive attention has been attracted to separating and enriching these vesicles from various biofluids.[128–134] Currently, the most common technique used to separate EVs from biofluid samples is differential centrifugation, which relies on differences in size and density between cells, cellular debris, and subgroups of EVs.[135, 136] Although this technique can achieve high purity of exosomes, its time-consuming, expensive, and often associated with complicated protocols, limiting the applications of EVs. Acoustic waves represent an emerging approach and offer versatility, high precision, and biocompatibility for manipulating cells and bioparticles. This section provides a summary of the recent progress in the acoustic handling of EVs including separation, enrichment, and transportation of EVs and nanovesicles.

To effectively separate EVs from biofluids, acoustic-based microfluidics has emerged as a simple and reliable method within miniaturized chips.[130, 137] Lee et al. developed an acoustic nanofiller system capable of isolating exosomes (as EVs with a diameter less than 200 nm) from the cell culture medium, utilizing size-dependent acoustic forces to selectively isolate nanovesicles with a yield greater than 90%.[138] To enable the direct separation of exosomes from undiluted blood samples without preprocessing, efforts have been made to design and optimize acoustic fields and acoustofluidic devices.[139] In this regard, the Huang group proposed a multiplex acoustofluidic platform to directly isolate exosomes from whole blood.[140] This platform integrated two sequential separation acoustofluidic modules utilizing the titled-angle standing surface acoustic waves (SAWs). The first module was used to remove cells and obtain enriched EVs, followed by the second module to remove other EV subgroups and ultimately achieve purified exosomes. They

demonstrated an exosome purity of approximately 98% and a yield of 82% from the mixture of purified exosomes and EVs. More recently, to isolate exosomes with high speed, yield, and purity, the Lee and Liu collaborative team developed an ultrafast-isolation system, called EXODUS, that allowed label-free purification of exosomes from different biofluids (Fig. 2a).[141] The authors used double-coupled harmonic oscillations into a dual-membrane filter configuration for vibrating nano-porous filters and generating acoustic streaming. Their results showed harmonic oscillations can reduce the fouling effect inside filters and promote the filtering process, indicating its promising potential for exosome research in life sciences and speedy practical translations in medicine.

Recently, acoustic strategies have demonstrated improved performance in the separation and transportation of exosomes when integrated with other physical fields or employing more powerful acoustic fields. For instance, Tayebi et al. combined electric and acoustic fields to sort subpopulations of EVs, particularly exosomes (diameter <200 nm) and EVs (diameter >300 nm).[142] By leveraging the synergistic effect of dielectrophoretic and acoustophoretic forces, the critical diameter for particle separation decreased significantly. In another study, the Huang group employed an acoustofluidic droplet centrifuge to enrich and separate nanovesicles simultaneously.[143] The use of slanted interdigital transducers (IDTs) to spin droplets resulted in the concentration of nanovesicles. The authors further demonstrated that two spinning droplets could attract nanovesicles with different sizes, enabling the separating subpopulations of EVs (Fig. 2b). Rather than using a simple acoustic field, the same group further presented a robust strategy known as the wave-pillar excitation resonance system.[144] This acoustic nanoscale separation method allowed for the rapid, single-step, high-purity (>96% small exosomes) fractionation of EV subgroups from biofluids without sample preprocessing (Fig. 2c). The most significant advantage of this acoustic platform was the cut-off size of the isolation as low as 50 nm. Beyond separation performance, sound waves can also activate and manipulate nanovesicles. Wu et al. developed a selective cell manipulation strategy using ultrasound-responsive gas-filled protein nanovesicles (GVs).[145] The authors demonstrated that the acoustic radiation force (ARF) acted differently on GVs and GV-expressing cells due to the difference in the acoustic contrast factor (Fig. 2d). They showed the powerful capabilities of GV-expressing cells in controllable transportation, bioprinting, and cell sorting.

Altogether, the isolation and interrogation of EVs and biomimetic nanovesicles could be enhanced with the support of sound waves. The use of acoustic radiation force and acoustic streaming-induced Stokes drug force has emerged as a promising strategy for achieving precise control of EVs. By leveraging the mechanical properties of these nanovesicles, precisely controlled acoustic fields have shown excellent performance in purifying EVs and transporting cells. These advances in the use of acoustic mechanical effects on EVs and nanovesicles have the potential to revolutionize the field of point-of-care diagnostics, enabling rapid and sensitive detection of diseases. Moreover, they can also be valuable tools for tissue engineering and disease management. Further research is expected to advance the understanding of the underlying physical mechanisms and unlock the full potential of EVs in life science and medicine.

### 3.2 Analysis of extracellular vesicles.

EVs and biomimetic nanovesicles have been widely used for various diagnoses such as biosensing and bioimaging at molecular, cellular, and tissue levels.[146–151] As natural carriers, EVs possess significant information that can reflect the physiological and pathological condition of an individual.[152] Moreover, biomimetic nanovesicles such as acoustically-responsive gas-filled nanovesicles (GVs) can carry different imaging and diagnostic probes. The detection and analysis of EVs and GVs are particularly useful in the early and personalized diagnosis of cancer. Wang et al. developed a SAW sensor that exhibited high sensitivity to detect exosomes (Fig. 3a). The SAW sensors used the piezoelectric effect to detect the phase delay of acoustic waves, and this acoustic sensing method has the advantages of label-free detection, easy operation, and real-time measurement.[153] Moreover, EVs and nanovesicles can also function as acoustic contrast agents for both sensings and in vivo imaging. Recently, Lu et al. demonstrated that GVs can produce strong contrast in magnetic resonance imaging (MRI) for clinical imaging diagnosis (Fig. 3b). Interestingly, the authors discovered that clustering-based GVs can be used as dynamic molecular sensors for multiplexed, functional, and genetically encoded molecular sensing and imaging.[154]

Biomimetic nanovesicles such as GVs possess stable molecular structures and scattering properties, making them an excellent candidate for bioimaging in clinics, disease monitoring, cellular function evaluation, and gene expression visualization. Natural EVs, without any modification, have demonstrated the ability to enhance ultrasound imaging through the scattering effect (Fig. 3c). Osborn et al. reported the first-ever echogenic exosomes that exhibited the unique acoustic responsiveness of traditional bubbles and the biocompatibility of nanoscale exosomes.[155] To explore the imaging ability of nanovesicles, a study reported a sialic acid (SA)-capped polymersome featuring a NIR profluorophore (pNIR) for lysosome activation-based optical and optoacoustic tumor imaging (Fig. 3d). Ultrasound signals generated from the reverse process of the ultrasound thermal effect, which is light-induced thermal expansion. The authors demonstrated that their pNIR@P@SA system can target tumor tissues for imaging-guided surgery.[156] Compared to optical methods, the sonic method allows for imaging at a much greater depth in tissues. To further improve the sensitivity and specificity of *in vivo* imaging, Hurt et al. reported that GV gene clusters in bacteria and mammalian cells exhibited a stronger acoustic scattering effect and produced non-linear signals from the background tissue (Fig. 3e). Their results demonstrated that GVs could non-invasively image *in situ* tumor colonization and gene expression in tumor homing therapeutic bacteria, track the progression of tumor gene expression and growth in a mouse model of breast cancer, and enable gene-expression-guided needle biopsies of a genetically mosaic tumor, showing non-invasive access to dynamic biological processes at centimeter depth.[157] In another study, GVs with acoustic scattering properties were used to ultrasonically image gene expression in mammalian cells with high spatial and temporal resolution, as shown in Fig. 3f. The authors engineered intracellular air-filled protein nanostructures called GVs that produced ultrasound contrast. These GVs allowed visualization of cells at tumor sites at volumetric densities below 0.5% and enabled high-resolution imaging of gene expression in living animals.[158]

Together, sound waves have emerged as a powerful tool for improving the diagnostic applications of EVs and biomimetic nanovesicles. Its piezoelectric and scattering effect enables the non-invasive, real-time, high-resolution, and high-sensitivity sensing in early disease detection and imaging in deep tissues or organisms for visualizing genes, proteins, cell functions, and events. Powered by sound waves, EVs and biomimetic nanovesicles may offer promising potential for bioimaging in clinics, disease monitoring, cellular function evaluation, and gene expression visualization. The stable molecular structure and scattering property of biomimetic nanovesicles make them better than optical imaging for deep-tissue imaging under some conditions. Through accessing dynamic biological processes remotely and noninvasively at a centimeter depth, this method shows great potential to improve the sensitivity and specificity for in-vivo imaging. Thus, the comprehensive summary of ultrasound-responsive nanovesicle applications and deep understanding of the underlying physical principles would find broader utility in life science and clinical medicine. The development of new and innovative approaches using ultrasound-responsive nanovesicles could lead to significant improvements in the diagnosis and companion diagnostics of various diseases, ultimately improving patient outcomes.

## 4. Therapeutics

EVs and biomimetic nanovesicles are developed as carriers of therapeutics for drug delivery.[159–163] These vesicles are capable of encapsulating both therapeutics and nanomedicine into their lipid bilayer structures.[164] Moreover, the natural membrane of these vesicles exhibits high compatibility, long-term circulation, and cell-targeting performance in vivo, making them attractive for drug delivery applications.[165, 166] With the help of sound waves, engineered EVs and biomimetic nanovesicles can be efficiently transferred, delivered, and released on demand. These vesicles are not only useful as drug carriers but also have direct therapeutic potential for diseases such as cancer, obesity, neural disorder, and other diseases.[167–174] Sound waves play a critical role in activating these engineered vesicles and promoting the generation of therapeutic vesicles for disease treatment. This section summarizes the current state-of-the-art applications in drug delivery and direct therapeutics.

### 4.1 Generation of extracellular vesicles.

EVs are generated through a process that involves double invagination of the plasma membrane and the formation of intracellular multivesicular bodies (MVBs) containing intraluminal vesicles, which can be influenced by sonic irradiation (Fig. 4a).[10, 175] Increasing evidence indicates that the endosomal sorting complex for transport required (ESCRT) machinery promotes the formation of vesicles in late-endosomal MVBs.[176, 177] In particular, exosome biosynthesis contains a complete pathway from subcellular to cellular events and can be regulated by genetic, molecular, and protagonistic approaches. Studies have shown that low-intensity ultrasound (LIUS) can enhance the generation of exosomes by changing the expression of genes associated with exosome biosynthesis, such as Rab GTPases, which control the secretory pathway to fuse with the plasma membrane for releasing exosomes.[178] High-frequency ultrasound (HFUS) can also be used to promote the generation of exosomes from cells (Fig. 4b), due to HFUS-driven transient

reorganization of the lipid structure of the plasma membrane, enabling the recruitment of extracellular $Ca^{2+}$. Furthermore, the calcium-initiated assembly of ESCRT accessory proteins can orchestrate the cascade of events that lead to the generation of exosomes.[179] In addition to upstream regulation of exosome-related pathways, sound waves can also directly change the function of specific proteins to regulate exosome production. For instance, the actin cytoskeletal regulatory protein cortactin promotes exosome secretion by modulating branched actin dynamics, which controls multivesicular late endosomes docking on the plasma membrane and exosome production.[180] The acoustic stimulus can accelerate the biosynthesis and secretion of the exosomes, providing an alternative source of exosomes for therapeutic applications.

### 4.2 Drug delivery.

Drug delivery using EVs and biomimetic nanovesicles can be significantly enhanced with the aid of sound waves. These vesicles are involved in the entire process of drug delivery, including drug loading, transportation, penetration, and release, for treating various diseases.[181–186] Wang et al. developed an acoustofluidic device for loading and encapsulation of drugs, and silica nanoparticles into exosomes using acoustic radiation force, acoustic streaming, and cavitation (Fig. 4c). They demonstrated that exosome-encapsulated nanomedicine exhibited exceptional efficacy in intracellular transport and inhibited tumor cell proliferation.[187] Inspired by the motor-like properties of biomolecules and organisms, gold nanowires coated with nanovesicles derived from red blood cells (RBCs) were utilized for on-demand transportation using ultrasound (Fig. 4d). The controllable propulsion phenomenon was achieved via acoustic streaming acting on the axially asymmetric concave ends, which allowed for efficient absorption and neutralization of RBC-targeted pore-forming toxins.[188] Additionally, ultrasound can temporarily open cellular and tissue barriers in the route of exosomal drug delivery, improving drug delivery efficacy. Sun et al. presented an ultrasound-assisted exosomal delivery of tissue-responsive mRNA to enhance gene therapy, utilizing ultrasound cavitation to minimize off-target effects in obesity therapy, as shown in Fig. 4e.[189] These findings highlight the potential of ultrasound in drug delivery, particularly in the context of exosomes and biomimetic nanovesicles.

Another key aspect of drug delivery is the control of drug release, which can be achieved by ultrasound simulation of sound-responsive EVs or biomimetic nanovesicles carriers. For example, Wang et al. fabricated an ultrasound-assisted erythrocyte membrane-derived hybrid nanovesicle drug delivery system (DOX/HMME@FA-NL) for enhancing tumor therapy (Fig. 4f). Due to the chemical effect of sound waves, reactive oxygen species (ROS) can be generated from the nanovesicle for oxidizing the unsaturated phospholipids, which caused the destruction of the membrane structure and controlled release of DOX.[190] Beyond the single function in drug delivery, more studies have reported novel nanovesicle systems with functions of drug loading, transportation, and release, simultaneously. Recently, Lu et al. developed a purely physical approach, using hydrodynamic forces induced by acoustic streaming, for controlling the loading and release of various drugs into and from nanovesicles (Fig. 4g). The authors provided a non-invasive method to control

material exchange across vesicle membranes, exhibiting the great potential in the cellular therapeutics.[74]

As a special tissue barrier, the blood-brain barrier (BBB) is a highly selective semi-permeable structure in the central nervous system that permits the passage of various nutrients, ions, and macromolecules.[191, 192] This critical structure functions to protect brain tissue from pathogens and potentially toxic substances, yet also poses a significant challenge to the treatment of brain diseases. The delivery of macromolecular therapeutics across the BBB remains a major obstacle in the development of effective treatments for central nervous system (CNS) disorders.[193–195] However, recent advancements in ultrasound-assisted nanovesicles have shown significant potential in enhancing BBB-crossing capacity and improving drug delivery.[196–198] Two underlying mechanisms contribute to the delivery enhancement of drug-loaded nanovesicles. Firstly, EVs have been demonstrated to actively cross the BBB through various uptake and transcytosis mechanisms, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, clathrin- and caveolae-independent endocytosis, and micropinocytosis, as shown in Fig. 5a.[199] Secondly, ultrasound has been shown to directly increase drug permeability to the BBB, as shown in Fig. 5b. The use of HFUS and microbubbles has been shown to generate stable cavitation and microstreaming, leading to the transient disruption of BBB tight- and adherent junctions, such as vascular endothelial (VE)-cadherin, occludin, claudin-5, and zonula occludens-1 (ZO-1) accessory proteins.[200] For example, one work developed an HFUS/bubbles system to open BBB, enhancing the LNP-mediated mRNA delivery (Fig. 5c).[201] Recently, Rezai et al. showed a noninvasive strategy to open BBB by using HFUS for treating Alzheimer's disease. The authors developed an ultrasound system that contained a helmet with 1024 ultrasound transducers. These transducers can be used to activate microbubbles and open BBB (Fig. 5d).[202] Together, the combination of sound waves and nanovesicles holds great promise in significantly enhancing drug permeability to the central nervous system and improving therapeutic effects on neurodegenerative diseases.

These research efforts and advances show the great potential of sound-assisted EV-based drug delivery. The non-invasive, deeper penetration, and precise regulation nature of sound waves allows for precise regulation of drug pharmacokinetics both *in vitro* and *in vivo*. Additionally, the new drug delivery approach may revolutionize traditional oral and injection medications, providing higher efficiency with fewer side effects. These findings highlight the importance of further exploring the potential of sound-assisted EV-based drug delivery for translational medicine.

### 4.3 Direct treatment.

EVs and biomimetic nanovesicles possess significant therapeutic functions, and recent advances in molecular engineering and synthetic biology have paved the way for biomolecular and cell-based therapeutics.[79, 203–206] For instance, research has demonstrated that biomolecules and cells can be modified to produce mechanical effects inside cells and tissues using ultrasound-induced inertial cavitation, as shown in Fig. 6a. Gas vesicles (GVs) can be converted into microbubbles with strong local mechanical effects, thereby disrupting cells and tissues. This approach has significant potential

for tumor-homing probiotic therapy. In addition to their physical therapeutic function, exosomes exhibit important biological properties such as regulation of cell-cell signaling and intracellular biomaterial transport.[207] Recently, Sheybani et al. demonstrated that focused ultrasound-induced hyperthermia could enhance the release of glioma-derived extracellular nanovesicles (Fig. 6b). Their findings showed that ultrasound-triggered release of nanovesicles could invoke a key signature of innate immune activation.[208] In another study, researchers demonstrated the potential of ultrasound in treating neurodegenerative diseases (Fig. 6c). They utilized ultrasonic mechanical stimulation to enhance the generation of exosomes from astrocytes, which provided neuroprotective effects and reversed oligomeric amyloid-β-induced neurotoxicity *in vitro*. Moreover, these exosomes facilitated the clearance of amyloid-β plaques *in vivo* when combined with focused ultrasound-induced blood-brain barrier opening, indicating their potential in treating Alzheimer's disease.[209] Altogether, EVs and biomimetic nanovesicles powered by sound waves hold great potential in disease treatments.

## 5. Perspectives

The potential of EVs and biomimetic nanovesicles in diagnostics and therapeutics powdered by sound has been demonstrated. However, challenges remain in exploring the underlying biological fundamentals of EVs and biomimetic nanovesicles and in leveraging sound physics and systems for broader translational medicine applications. Biologically, to uncover more cellular communication abilities of EVs, there is an urgent need for the quantitative study of the biogenesis, trafficking, and cellular entry of EVs and/or biomimetic nanovesicles.[210–212] Moreover, It is promising to massively generate EVs for therapeutic purposes, but the mechanism is still unclear for enhanced EV generation by sound waves. Technically, standardized purification and analytical procedures should be developed to study exosomes, which may reveal their functional heterogeneity.[213–215] Due to the nano- to submicron-sized of these vesicles, the forces generated by sound waves must be strengthened by advancing the design and incorporating other physical and chemical methods to achieve a better manipulation of these vesicles.[216–218] If possible, well-designed sound beams may directly manipulate EVs *in vivo* for enhancing their diagnostic and therapeutic abilities.[219, 220] Clinically, the translational diagnosis and therapy using sound-powdered EVs and biomimetic nanovesicles are still challenging, mainly due to the variability of EVs biomimetic nanovesicles and the non-standard protocol in applying sound in vivo. For example, the precise detection of EVs in biofluids still hinders the development of the point-of-care diagnosis of various diseases in a precise and long-term manner. Finally, challenges remain in manipulating and decoupling multiple effects induced by sound waves to achieve diagnostics and therapeutic purposes of EVs and biomimetic nanovesicles.

The future of EVs and biomimetic nanovesicles powdered by sound waves is expected to further explore the fundamental life sciences and translational medical applications in several aspects: (1) One area of advancement is the development of point-of-care diagnostic technologies through the innovation and integration of various EV isolation and characterization approaches. For example, acoustic and acoustofluidic EV handling devices can be integrated with characterization techniques, such as multimodal sensors, to enable real-time detection and/or analysis of isolated EVs.[221, 222] Furthermore,

these devices can be miniaturized and integrated into portable lab-on-a-chip platforms, enabling the development of new disease detection methods as well as new approaches for monitoring disease progression in clinics. (2) Another advancement is the EV-based biomarker discovery for exploring disease etiology. The innovation of sound-powdered EV handling may address the current sample preparation and processing challenges for current multi-omics technologies for precise investigations of the biomarkers carried by EVs from patients. (3) Another future direction is the further development of ultrasound-targeted delivery of EVs to specific tissues or organs in the body for disease treatment. [160, 223] Further efforts are required to design a better acoustic field and novel ultrasound-responsive multifunctional EVs and biomimetic nanovesicles for improving the safety, specificity, efficiency, and convenience of current therapy approaches. (4) The integration of artificial intelligence (AI) has the potential to further improve the sound powdered diagnostic and therapeutic performance of these vesicles by providing deeper insights into precision medicine and personalized therapy.[224–228] Specifically, AI algorithms can optimize the design of acoustic fields and biomimetic nanovesicles, advance the system and procedure of the sound-powered EVs/biomimetic nanovesicles technologies, and facilitate the understanding of fundamental EV biology for various diseases. In conclusion, the combination of sound waves and EVs/biomimetic nanovesicles holds vast potential for future applications in the fields of life science and medicine.

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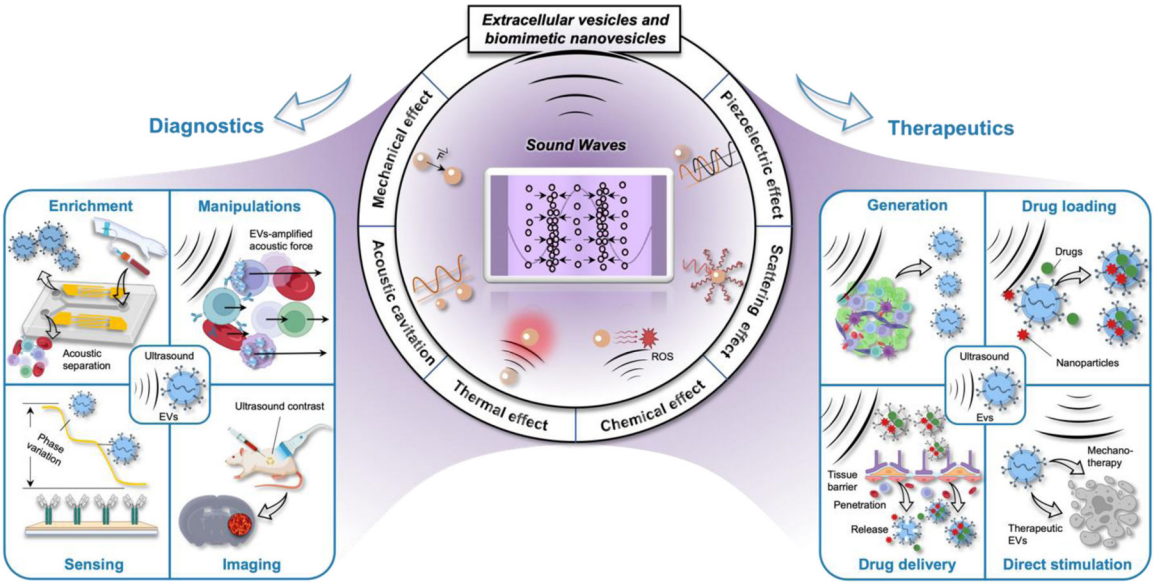


**Fig 1. Overview of exploiting sound waves for applications of extracellular vesicles and biomimetic nanovesicles.**

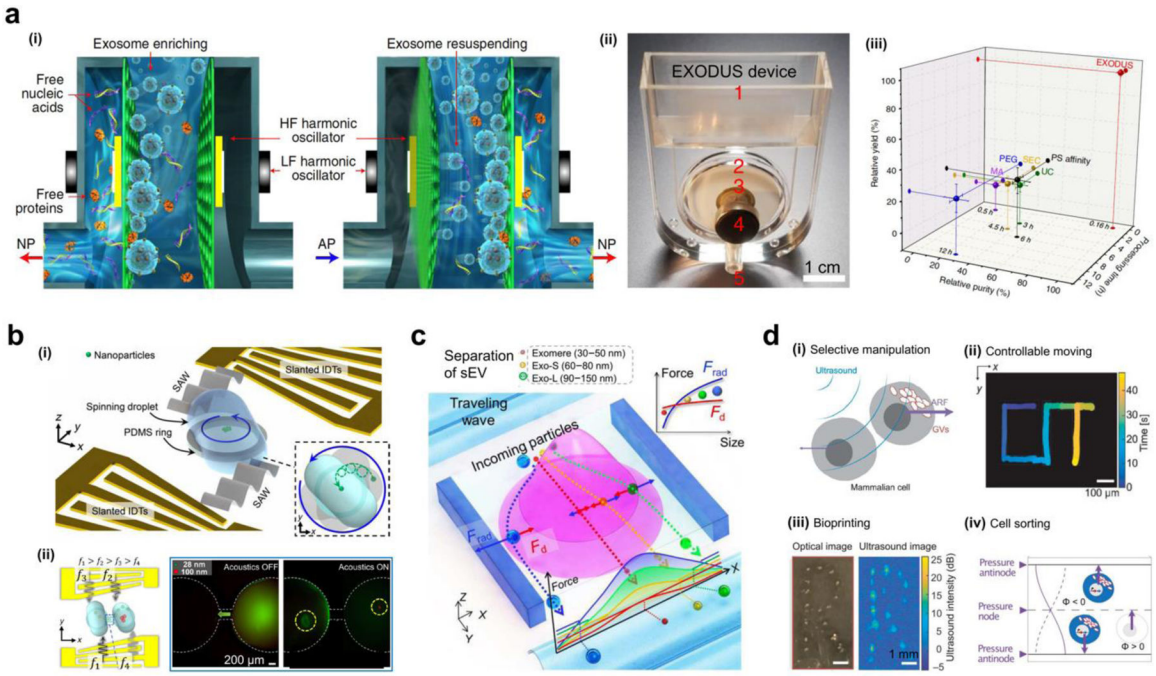


**Fig 2. Manipulations of extracellular vesicles.**

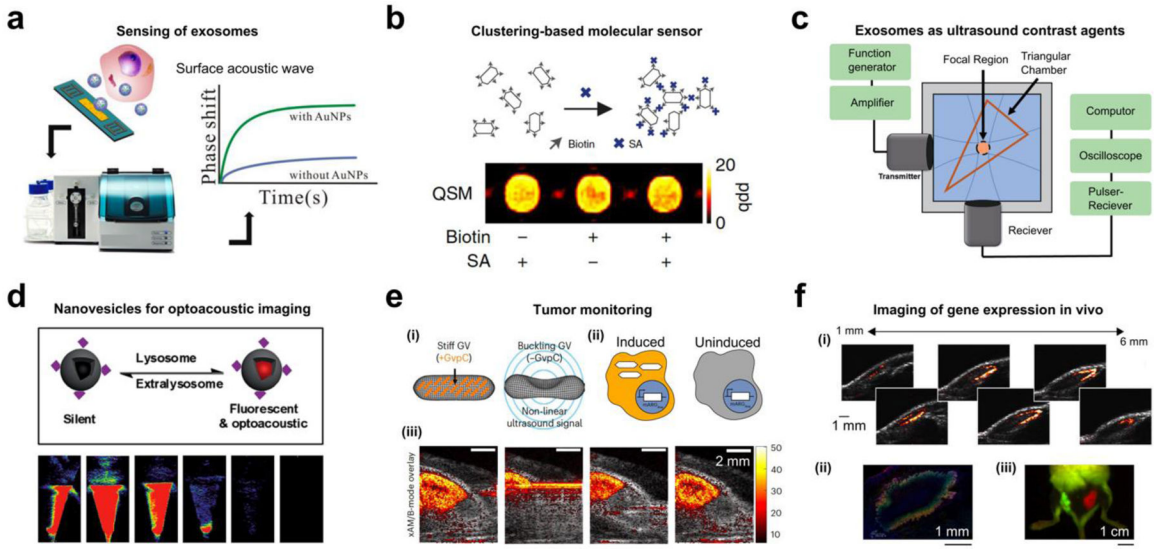


**Fig 3. Detection and analysis of extracellular vesicles.**

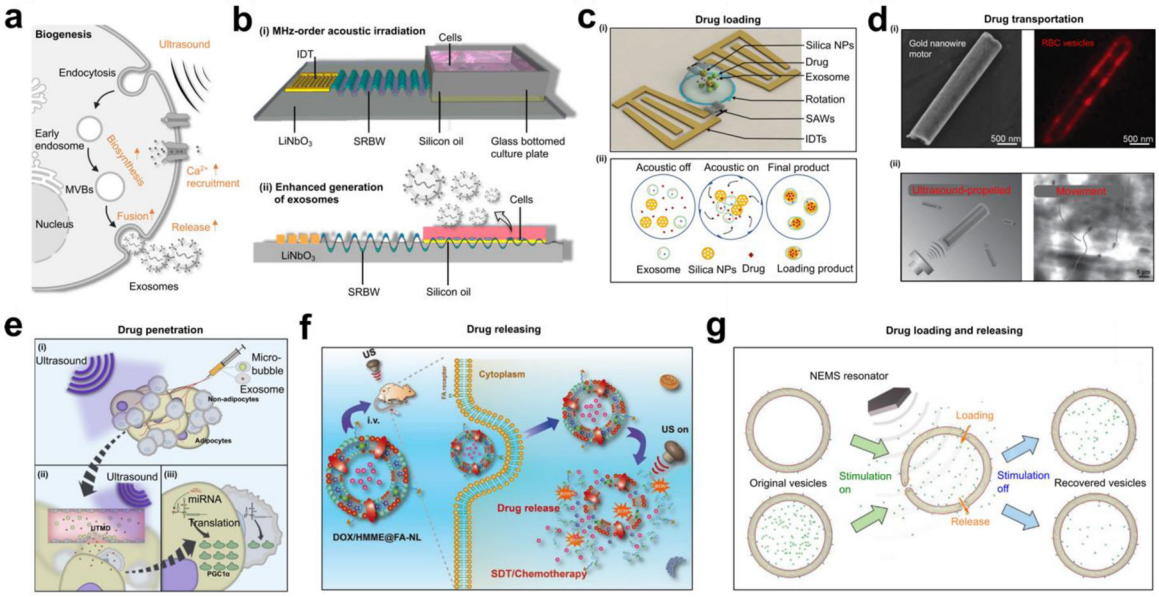


**Fig 4. Extracellular vesicles and biomimetic nanovesicles as excellent drug carriers.**

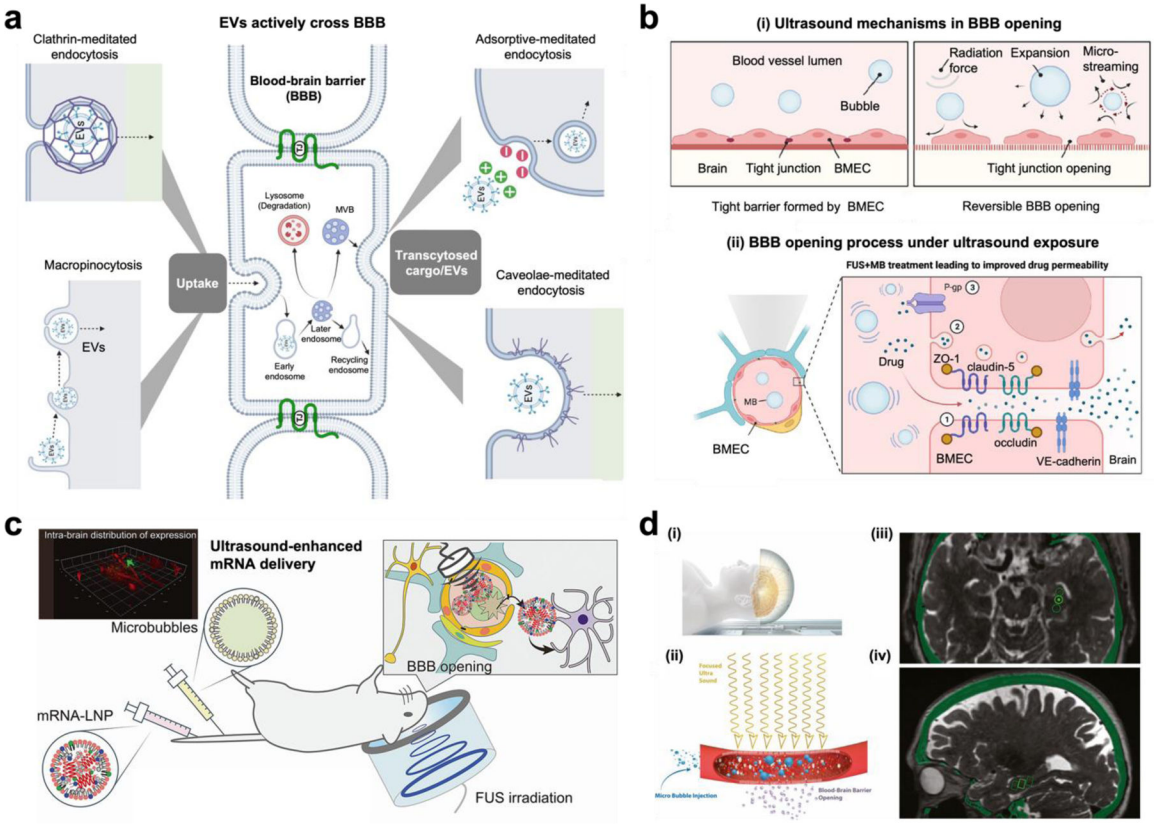


**Fig 5. Vesicles cross the BBB enhanced by sound waves.**

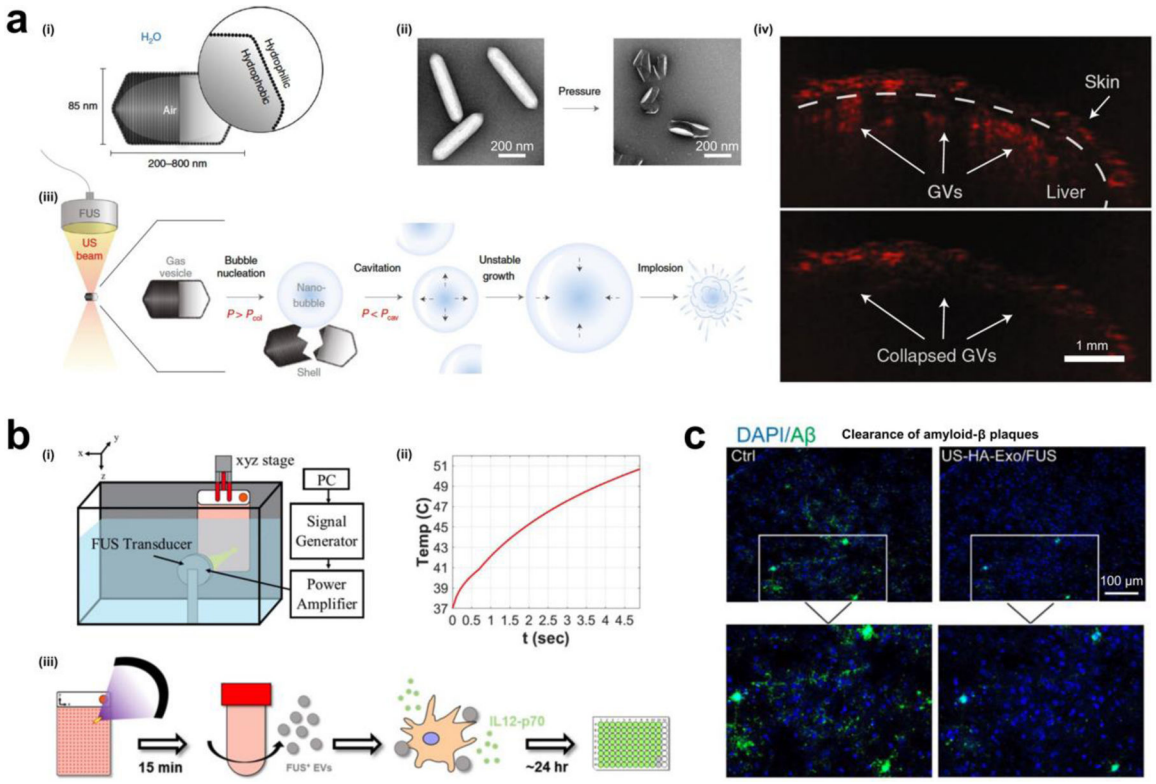


**Fig 6. Direct treatment using ultrasound.**