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Acoustic technologies for the orchestration of cellular functions for therapeutic applications

Ye He[1], Jianping Xia[1], John D. H. Mai[2], Neil Upreti[1], Luke P. Lee[3,4,5,6,7]*, Tony Jun Huang[1]*

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Mechanical forces constantly stimulate cellular functions and influence their response behaviors. Similar to how an orchestra's music synchronizes an audience, acoustic technologies have emerged as precise, contact-free tools to study cellular responses. These platforms generate forces at appropriate length and frequency scales, enabling precise interactions with cells. Recent advancements highlight their potential for regulating cellular functions, revealing both therapeutic promise and the need for further biochemical exploration. This review summarizes the progress in using acoustic technologies to orchestrate cellular functions in vitro through mechanical stimulation. We first introduce the main categories of acoustic platforms and their working principles in cellular research. Subsequently, we explore the fundamental mechanisms linking acoustics to specific cellular interactions. We then review recent applications of these technologies in precisely modulating cellular functions for therapeutic purposes. Last, we discuss strategies to enhance their performance and efficacy, along with their potential integration with other biomedical tools.

INTRODUCTION

Although the regulation of cellular functions by mechanical stimulation is often perceived as an emerging field, insights into cellular mechanosensing and the corresponding response have been recorded for over a century. In 1917, Thomson (*1*) pioneered the concept of considering physical laws and mechanics as key determinants of the shape and structure of living organisms, alongside evolution. However, technological limitations prevented the experimental validation of many theoretical concepts. In the past few decades, the study of mechanical influences on cells has experienced great progress, fueled by advancements in microscopy, micro/nanofabrication technologies, and the introduction of innovative analogs to mechanical tools such as magnetic and optical tweezers (*2*–*8*). As a result, mechanical inputs are now considered as essential biochemical signals in regulating and governing the life cycle of cells and organisms, affecting processes from embryonic development to cancer and aging (*9*–*11*). Cells can perceive, internalize, and respond to external mechanical cues and alter cellular functions accordingly.

In contrast to the gradient diffusion of biochemical factors, mechanical signals are more direct and rapid. Thus, they can accurately and precisely regulate cellular functions, which holds great importance in stem cell maturation, immune activation, and tumor therapy (*12*–*14*). Internal forces, such as membrane tension and cellular tensegrity, play an integral role in helping cells adapt to changes in the extracellular matrix (ECM), dynamically maintaining or adjusting their morphology (*15*). External forces from adjacent cells or environments, such as shear and compressive forces, remarkably influence cell adhesion, proliferation, and differentiation and have even been shown to play a role in paracrine signaling (*10*, *16*, *17*). Therefore, effectively scaling and using mechanical tools to manipulate mechanical signals is essential for optimizing cellular functionality, underscoring its significance for biomedicine.

Acoustic technologies are a rapidly growing area of biomedical research that have proven to be excellent toolkits for mechanically regulating cellular functions (*18*–*23*). Acoustic wave generation can be precisely controlled by adjusting the input power and working frequencies, allowing for the creation of programmable wave patterns (*24*–*26*). In addition, the interaction of acoustic waves with cells occurs in a contactless and biocompatible manner (*27*). These features are advantageous for cell biology studies as they minimize the possibility of external interference and contamination. This also results in high reproducibility at force magnitudes conducive to cellular modulation, enabling direct correlations between mechanical stimuli and cellular outcomes. In addition, the elimination of labeling removes the risk of functional changes in the cell that can arise from biochemical fouling, alterations in culture conditions, or systematic errors due to the excessive use of intermediate materials (e.g., polymer substrates or magnetic beads) (*28*). Moreover, the high biocompatibility of acoustic waves, along with known transport properties in aqueous media, makes them particularly well suited for in vivo applications (*29*–*33*). Because acoustic waves can penetrate thick tissues, they can generate therapeutic effects in various target organs or cell populations of interest.

Acoustic technologies have been demonstrated as effective tools for controlling various biological behaviors both in vitro and in vivo (*34*–*38*). Beyond the direct application of different types of ultrasound modalities on tissues or organs for therapeutic interventions like tumor ablation or wound healing, many studies have already developed in vitro analysis platforms to explore the regulatory effects of specific acoustic actions on cellular functions (*39*–*42*). In this review, we discuss the recent advances in acoustic technologies focused on modulating cellular functions in vitro. We also highlight the feasibility and rationale of regulating cellular functions via mechanical stimulation and explore underlying mechanisms using

[1]Thomas Lord Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC 27708, USA. [2]Alfred E. Mann Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA. [3]Renal Division and Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Harvard University, Boston, MA 02115, USA. [4]Department of Bioengineering, University of California, Berkeley, Berkeley, CA 94720, USA. [5]Department of Electrical Engineering and Computer Science, University of California, Berkeley, Berkeley, CA 94720, USA. [6]Department of Biophysics, Institute of Quantum Biophysics, Sungkyunkwan University, Suwon, Korea. [7]Department of Chemistry and Nanoscience, Ewha Womans University, Seoul, Korea.
*Corresponding author. Email: lplee@bwh.harvard.edu (L.P.L.); tony.huang@duke.edu (T.J.H.)

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various acoustic technologies, emphasizing their connection to therapeutic effects. In addition, this review provides insights into improving existing acoustic technologies to optimize their performance and therapeutic effects, including potential synergies with other biomedical tools.

COMMONLY USED ACOUSTIC TECHNOLOGIES IN BIOMEDICAL RESEARCH

Acoustic waves are versatile and support a variety of therapeutic applications. This versatility has led to the development of a rich catalog of acoustic technologies tailored to optimize therapeutic outcomes (*32*, *33*). These acoustic technologies are categorized based on frequency, sound intensity, and excitation method. In terms of frequency, acoustic technologies can be broadly classified into three categories: high-frequency ultrasound (>10 MHz), low-frequency ultrasound (20 kHz to 10 MHz), and ultralow-frequency (<20 kHz) sound waves (*43*–*46*). In addition, these technologies can be further grouped based on sound intensity, distinguishing between high-intensity and low-intensity acoustic waves (*46*, *47*). Furthermore, depending on the excitation method used, acoustic technologies can be categorized as continuous waves or pulse waves (*48*, *49*). Here, we generally use the term bulk waves to refer to continuous or pulse waves generated by bulk piezoelectric transducers. Similarly, the terms traveling surface wave, standing surface wave, or surface acoustic waves (SAWs) generally correspond to continuous or pulse wave generation by lithographically patterned and micromachined acoustic transducers. Figure 1 briefly summarizes some of the representative acoustic technologies, emphasizing which features are suited for each application.

Bulk acoustic waves

Bulk acoustic waves, generated by resonating an entire piece of piezoelectric material, play a pivotal role in cell biology research (*50*). Typically, these waves are produced using specialized ultrasound transducers equipped with electrodes on a piezoelectric substrate. Applying an alternating current effectively applies an alternating voltage to the transducer, which induces mechanical deformation due to the inverse piezoelectric effect, resulting in mechanical

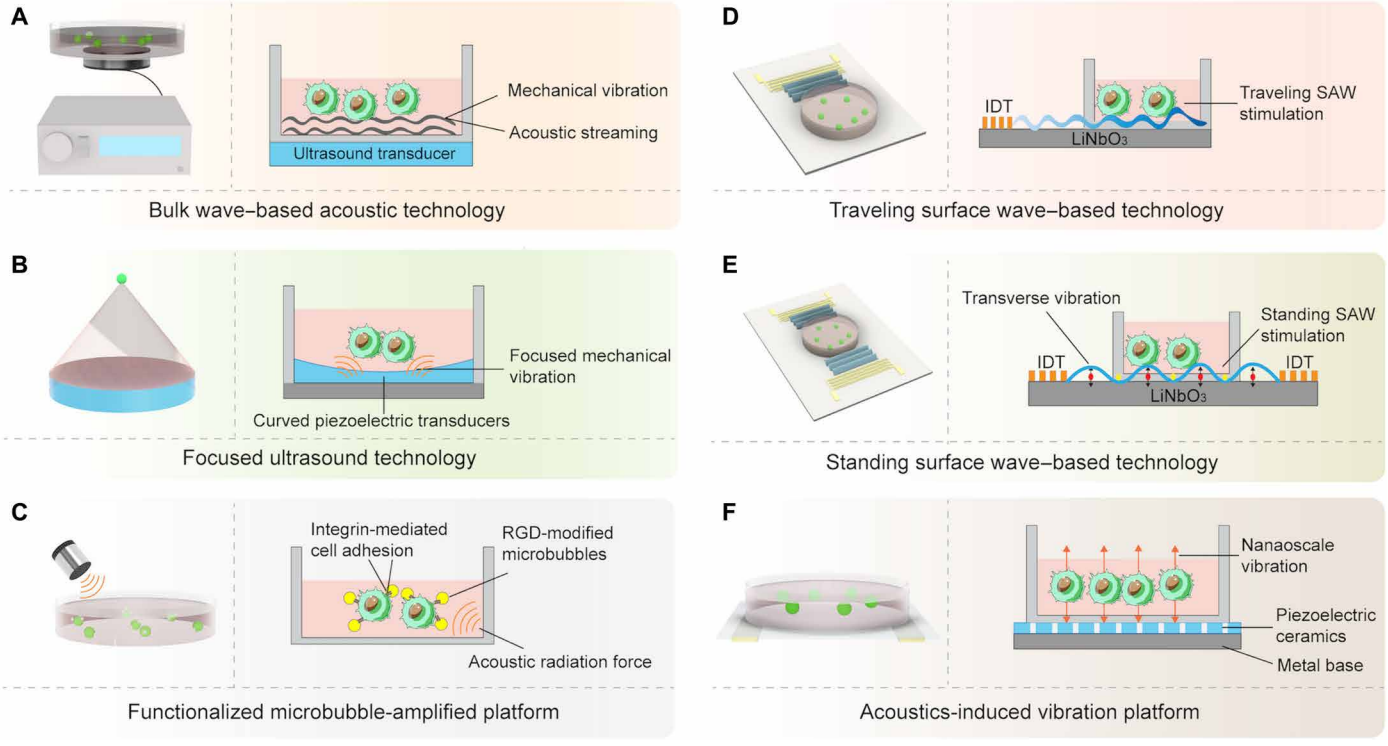


Fig. 1. Commonly used techniques to generate acoustic waves in biomedical research. (**A**) Bulk wave–based acoustic technology is the traditional and most widely used acoustic technology in biomedical studies. These acoustic waves are typically continuous waves produced using ultrasound transducers equipped with electrodes on a piezoelectric substrate. After applying an alternating voltage to the transducer, a mechanical deformation can be induced through the inverse piezoelectric effect, resulting in the generation of mechanical vibrations. (**B**) Focused ultrasound technology generates high-intensity ultrasound at the focus point by a single bowl-shaped piezoelectric ceramic transducer or an array of bowl-shaped transducers. This technology is widely applied in tumor ablation and neural stimulation. (**C**) The functionalized microbubble-based platform is a specialized acoustics-assisted method to induce cell membrane–cytoskeleton vibration by transmitting acoustic radiation forces to cells via integrin-RGD interactions (*70*, *71*, *117*). (**D** and **E**) A surface wave is commonly a pulse wave generated by applying an alternating voltage signal with a specific frequency to the interdigitated transducer (IDT), which propagates along the surface of a solid material, such as quartz or lithium niobate ($LiNbO_3$). One single IDT generates a traveling surface wave and creates acoustic streaming. One or two pairs of IDTs can generate a standing surface wave and exert a powerful acoustic radiation force by leveraging the gradient distribution of acoustic pressure and velocity. (**F**) The acoustics-induced vibration platform achieves mechanotransduction to cells by placing piezoelectric ceramics under a ferrous actuating top plate. Then, the input electric signal induces the plate to vibrate with a nanoscale peak-to-peak amplitude that produces nanoscale displacements (*74*, *213*). This figure was generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 4.0 unported license (https://creativecommons.org/licenses/by/4.0/deed.en) (Servier; https://smart.servier.com/)

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vibrations (*51*). The piezoelectric transducer typically operates in one of three modes: thickness, radial, and lengthwise (*52*). For cell biology applications, thickness mode is commonly used as it vibrates out of plane and efficiently delivers waves at ultrasound frequencies into the cell culture medium (*53*). In this case, the transducer's frequency is inversely proportional to its thickness, meaning a thinner transducer resonates at a higher frequency. In this context, operating frequencies typically range from 20 kHz to slightly above 10 MHz. It is worth noting that these traditional transducers are seldom designed to work at frequencies higher than 15 MHz due to manufacturing limitations (*54*). Exceptions such as membrane-type piezoelectric transducers with much higher frequencies have been proposed, but they are generally used in sensing applications (*55*).

Bulk wave transducers typically produce low-power ultrasound waves (like the prevalent low-frequency continuous/pulse ultrasound). To address this, the thickness of the transducers is frequently adjusted and formed into an arc (*56*). This modification allows for the modulation and focusing of ultrasound wave fronts into a highly intense beam, with power outputs ranging from several watts to more than 100 W. Currently, the most commonly used bulk ultrasound technique for cell stimulation applications is the low-intensity pulsed ultrasound, as the low-intensity (typically <3 W/cm^2) and pulsed waveforms can minimize potential mechanical damage and unwanted induced thermal effects to cells (*57*).

Surface acoustic waves

SAWs, also known as Rayleigh waves, are mechanical waves that propagate along the surface of solid materials, such as quartz or lithium niobate (*58*). These waves travel parallel to the surface and are confined to a thin layer just beneath the material's surface. Interdigitated transducers (IDTs) are often used to generate SAWs (*59*). These transducers contain closely spaced metal electrodes deposited onto a piezoelectric substrate and lithographically patterned. When an alternating voltage signal with a specific frequency is applied to these electrodes, a periodic electric field is generated, which induces periodic mechanical strain in the piezoelectric material. These changes in strain result in the creation of SAWs. Various types of IDTs have been developed, such as plane wave, slanted, focused, and chirped (*60*).

SAW devices are widely used in cellular biology research for several compelling reasons. Specifically, SAWs can be highly effective within fluidic systems—a field known as acoustofluidics (*19*). This approach enables precise manipulation of fluids and particles such as cells and extracellular vesicles, exerting great influence on cellular behaviors such as viability, proliferation, stress response, and functionality. In addition, SAWs are known for their gentle treatment of cells and biomolecules, minimizing the risk of cellular damage or protein degradation (*61*). In the context of cell biology research, SAW devices generally operate at high frequencies, ranging from 10 to 400 MHz (*62*, *63*). SAWs exist in two primary modes: traveling and standing (*64*). Traveling SAWs are typically generated by a single IDT and propagate into the surrounding fluid, creating acoustic streaming. In such cases, the acoustic radiation force is often outweighed by the drag force due to wave attenuation. On the other hand, standing SAWs are usually generated using one, two, or more than two pairs of IDTs. They leverage the gradient distributions of acoustic pressure and wave velocity to exert a powerful acoustic radiation force, making them ideal for precise cell manipulation. In addition, their wavelengths, typically ranging from 10 to 400 μm, are matched to the length scales of cells (*63*).

Acoustic cavitation

Acoustic cavitation refers to the formation and subsequent abrupt collapse of bubbles within a liquid when exposed to intense ultrasound waves (*65*). When high-intensity sound waves traverse through a liquid, tiny gas-filled microbubbles emerge during the low-pressure phase of the wave. These microbubbles progressively grow as the pressure drops. During the high-pressure phase of the acoustic wave, there is a rapid increase in pressure, causing the bubbles to collapse with tremendous force. This collapse results in the generation of shockwaves, microjets, and the localized generation of extremely high temperatures and pressures. One notable application of high-intensity focused ultrasound (HIFU) with acoustic cavitation is its use in the precise targeting and treatment of specific tissues or tumors (*66*). This targeted approach leads to tissue destruction or ablation, facilitated by the intense localized heat generated during the process. In addition, cavitation has the capacity to create transient pores in cell membranes, enabling the localized enhancement of therapeutic drug delivery through translocation, and inducing cellular function changes (*67*).

Functionalized microbubbles

In addition to using acoustics to directly stimulate cells, a force amplifier platform that uses functionalized microbubbles has been developed and demonstrated the feasibility of regulating cell functions (*68*–*71*). Similar to acoustic cavitation, membrane-bound gas microbubbles can serve as an acoustic radiation force "amplifier" to strengthen the direct ultrasound stimulation of cells. The imbalance of acoustic impedance between the gas inside of the microbubbles and the surrounding liquid can amplify the resulting acoustic excitation, which pushes microbubbles toward cells and induces a secondary mechanical force (*70*). Typically, lipid microbubbles are used and functionalized with molecules such as ECM-mimicking RGD peptides, allowing them to covalently attach to cells through the RGD-integrin interaction. After applying acoustic radiation forces to the functionalized microbubbles, the microbubbles can rapidly transmit forces to cells through integrin signaling and induce changes in cellular functions.

Acoustic-induced vibrations

Acoustic-induced vibration of a substrate is another alternative way to transmit forces to cells in a controlled manner. By placing a piezoelectric ceramic plate under a cell culture substrate, an applied input electric signal can induce the plate to vibrate through the reverse piezoelectric effect. The vibration scale can be easily tuned by adjusting the input frequency and amplitude. For example, a nanoscale vibration can be driven by a 1-kHz driving frequency (*72*–*74*). This nanoscale vibration can further induce cell vibration and alter cellular behaviors through increased adhesion-driven cytoskeletal tension.

MECHANISMS OF ORCHESTRATING CELLULAR FUNCTIONS USING ACOUSTICS

Acoustic waves propagating in a cell culture medium can induce rhythmic pressure changes and generate a "massage effect" on cells (*75*). This massaging effect can cause the rearrangement of the cytoskeleton, changes in cell volume, or associated increases in membrane permeability. The intensity and applied area of this effect can be finely tuned with different acoustic technologies, and it can remarkably affect cellular behaviors such as proliferation, migration, differentiation, or even apoptosis (*50*, *76*). Different forms of acoustics can

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regulate cellular functions through various mechanisms that involve the modulation of cellular signaling pathways (*77*, *78*). In addition to effects that can directly cause irreversible structural damage, such as thermal and lipolytic effects, acoustics can beneficially regulate cellular functions efficiently by cell surface receptors/channels, integrin-mediated mechanotransduction pathways, and sonoporation effect, which has been reported by extensive studies (*68*, *79*–*87*). Here, we briefly summarize the main mechanisms in Fig. 2 to explain how acoustics can effectively regulate the functions of different cell types.

Activating cell surface receptors/channels using acoustics

Various cell surface receptors and channels, including mechanosensitive ion channels, voltage- and ligand-gated channels, and G protein–coupled receptors, have been shown to be effectively activated by ultrasound waves (*79*, *80*, *82*–*87*). Among them, mechanosensitive ion channels are the most widely studied. Mechanosensitive ion channels are a class of ion channels activated by mechanical forces, such as stretching and compression of the cell membrane, by an acoustic radiation force or a drag force (*88*–*90*). Mechanosensitive ion channels are integral membrane proteins embedded in the cell membrane and serve as molecular sensors for mechanical stimuli.

There are two conventional models that explain the working principles of the mechanosensitive ion channels: the force-from-lipids model and the tethering to the ECM or cytoskeleton model (*91*). For the force-from-lipids model, in the "resting state," the channels are in a closed or inactive conformation. The lipid bilayer

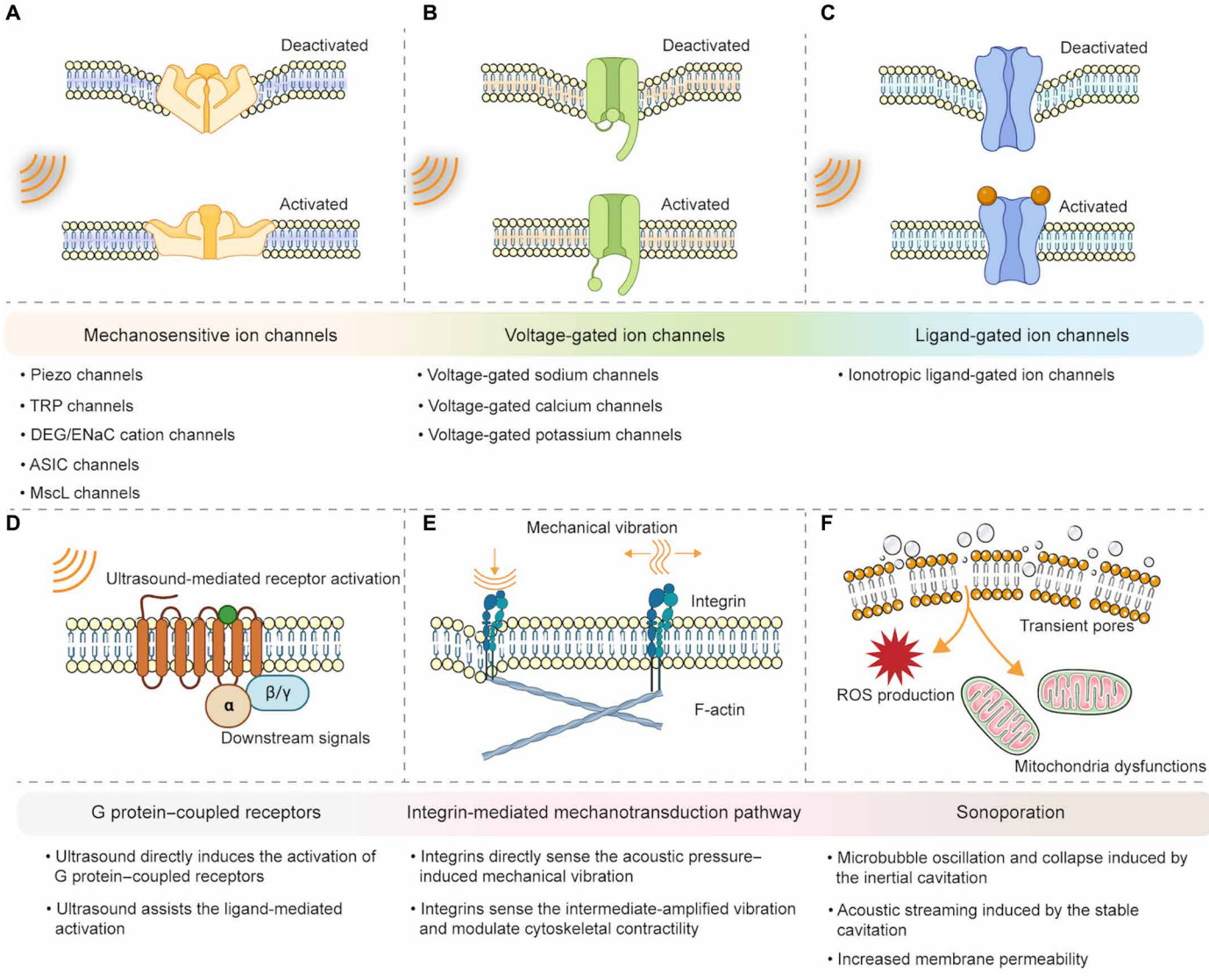


Fig. 2. Basic mechanisms for orchestrating cellular functions using acoustics. The conformational changes of (**A**) mechanosensitive ion channels; (**B**) voltage-gated ion channels; and (**C**) ligand-gated ion channels can be regulated by applying acoustic stimulation. (**D**) Ultrasound can directly induce the activation of G protein–coupled receptors (*79*) or assist the ligand-mediated activation of G protein–coupled receptors (*87*). (**E**) Mechanical vibrations transmit acoustic forces to the cytoskeleton and influence cellular functions by the integrin-mediated mechanical transduction pathway. (**F**) Acoustics-induced sonoporation results in cellular function changes by creating transient pores on the cell membrane and influencing mitochondria activity. This figure was generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 4.0 unported license (https://creativecommons.org/licenses/by/4.0/deed.en) (Servier; https://smart.servier.com/). Created in BioRender. Ma, Z. (2025); https://BioRender.com/gmnx56i.

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and surrounding molecular structures maintain mechanical tension across the membrane, keeping the channel closed. When the cell membrane senses the mechanical stimuli, the local transbilayer pressure profile shifts, inducing conformational changes and the gating movement of the channels. These channels are then transiently switched to an "open state" from the resting state and allow specific ions, such as calcium, sodium, and potassium, to pass through the channel pore, leading to ion flux across the cell membrane. The ion flux results in alterations in the membrane potential, the generation of electrical signals, and the induction of downstream cellular signal pathways. In the tethering to the ECM or cytoskeleton model, the mechanosensitive proteins are tethered to the ECM or the cytoskeleton. These tethers convey forces directly to the ion channels. Mechanosensitive ion channels are ubiquitously present and active in diverse tissues and organs, such as the central and peripheral nervous systems, heart, bones, and lungs (*92*). They play important roles in the sensations of touch, pain, and temperature change. Hence, mechanosensitive ion channels are the main regulators of sensory transduction, mechanotransduction, and cellular homeostasis.

There are several typical mechanosensitive ion channels, such as piezo channels, degenerin/epithelial sodium channels, acid-sensing ion channels, mechanosensitive channels of large conductance and mechanosensitive channels of small conductance channels, and transient receptor potential (TRP) channels (*91*). Previous studies have confirmed that many of the mechanosensitive ion channels can be activated by acoustic waves, such as two-pore potassium channels, TRP channels, and piezo channels (*93*, *94*). Among them, piezo channels (including Piezo1 and Piezo2) are the most extensively studied in relation to acoustic stimulation response, especially the shear stress induced by acoustic streaming. The effectiveness of piezo channels can be seen in many cell types, such as adult stem cells, tissue progenitor cells, endothelial cells, and neurons (*95*, *96*). The TRP family, composed of channels such as TRP-4, TRPC1, and TRPP2, is another example of channels that can be rapidly activated by acoustics (*88*, *97*). The brief exposure time required for TRP protein activation (10 to 100 ms) suggests the high biocompatibility of acoustics as a trigger.

In addition to mechanosensitive ion channels, the conformational changes of voltage-gated ion channels and ligand-gated ion channels and the activation of G protein–coupled receptors by acoustic stimulation have also been demonstrated (*79*, *82*–*87*). For voltage-gated ion channels and ligand-gated ion channels, acoustic forces help mediate the conformational changes and ion influx to induce intracellular responses. For the activation of G protein–coupled receptors, acoustic forces can directly up-regulate the expression of these receptors (*79*). Alternatively, acoustic forces also assist in ligand-mediated activation. For example, low-intensity pulsed ultrasound can induce the release of ATP/purines to activate the P2Y receptor and improve the proliferation of osteoblasts (*87*).

Activating integrin-mediated mechanotransduction pathways using acoustics

In addition to cell surface receptors/channels, acoustic forces can be directly transmitted through integrin-mediated mechanotransduction pathways (*98*, *99*). Integrins are transmembrane receptor proteins that connect the ECM to the cytoskeleton (including actin and actomyosin). By binding to the ligands within the ECM, such as collagen and fibronectin, integrins facilitate the formation of complex structures known as focal adhesions (*100*). Focal adhesions help cells to attach to the ECM and facilitate the transmission of mechanical forces and biochemical signals. Several signal pathways are associated with integrin-mediated mechanical transduction, such as Rho-associated protein kinase (ROCK), transforming growth factor–β1 (TGF-β1)/mammalian target of rapamycin (mTOR), and mitogen-activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK) (*101*–*103*).

Typically, integrin-mediated mechanotransduction pathways transmit acoustic forces through focal adhesion–mediated matrix-cell interactions. For example, when functionalized lipid microbubbles are attached to cells through integrin binding, the acoustic stimulation induces cytoskeleton contractility and activates the classic Hippo pathway through Yes-associated protein (YAP) signaling (*69*). Researchers recently found that integrin can directly sense acoustic stimulation and modulate downstream signals. For example, the integrin-mTOR signaling pathway is activated by ultrasound and facilitates the TGF-β1–induced chondrogenesis of mesenchymal stem cells (MSCs) in bone marrow (*102*). In addition, the mechanosensitive focal adhesion protein vinculin was also reported to play a key role in responding to external acoustic stimulation (*104*).

Acoustic-induced sonoporation

Except for regulating biochemical molecules, acoustics-induced sonoporation can also directly influence cellular functions (*67*, *105*, *106*). Sonoporation refers to the temporary disruptions of the cell membrane by acoustic energy (*107*). This process involves using acoustics to form transient pores or cavitation bubbles on the cell membrane, which increases the permeability of the cell membrane. Engineered micro-sized bubbles are incubated with cells to serve as the cavitation site nuclei and to improve the sonoporation efficiency.

Typically, sonoporation is used in drug delivery research to facilitate the uptake of nanoparticles and biochemical molecules by cells (*108*). However, recently, an increasing number of studies have claimed that sonoporation can also induce changes in cellular function. For example, enhancements in membrane permeability and rapid cytoskeleton disassembly after sonoporation are effective in regulating cell cycle phases (*106*). Disruption of the cell membrane affects mitochondrial functions and initiates cell apoptosis. The oscillation of microbubbles can also enhance the production of reactive oxygen species (ROS) (*109*, *110*).

CONTROLLING CELLULAR FUNCTIONS USING ACOUSTICS

In vitro investigation of cellular mechanobiology provides a controlled and dynamic platform to decipher the intricate interactions between cells and their mechanical surroundings. This burgeoning field holds immense promise in shaping the landscape of therapeutic applications by revealing the mechanical cues that govern cellular behavior. Acoustic technologies are well positioned to rigorously study the influence of mechanical forces on specific cellular functions. To date, the cellular functions under various therapeutic scenarios have been successfully orchestrated by many acoustic actuation modalities, which supports an optimistic future for clinical applications of acoustic technologies. Here, we introduce the current progress in using different acoustics-based platforms to orchestrate the biological effects and therapeutic functions of several important cell types and their biological effects, such as stem cells, tissue progenitor cells, immune cells, and neural cells. Additional details can be found in Table 1,

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Table 1. Summary of acoustic technologies for controlling various cellular functions.

Acoustic technologies	Parameters	Cell source	Cellular functions	Biological mechanisms	Refs.
Orchestration of stem cells					
Traveling surface acoustic wave-based platform	20 MHz frequency; 24, 31, and 39 V of RF amplitudes; pulse mode with a burst period of 1 ms, 200 pulses per burst	Human embryonic stem cells	Neural differentiation	Ion channel activity, receptor ligand, and transmembrane transporter activity	(*115*)
Functionalized microbubble-based platform	1 MHz frequency; acoustic pressure from 0.03 to 0.04 and 0.05 MPa	Human embryonic stem cells	Loss of pluripotency and epithelial-mesenchymal transition	Increased cellular contractility, activated focal adhesion kinase, inhibited YAP nuclear localization	(*68*)
Low-intensity pulsed ultrasound	3 MHz frequency; 20–50 mW/cm^2 of intensity	Rat mesenchymal stem cells	Chondrogenic differentiation	Regulation of autophagy	(*116*)
Standing surface acoustic wave-based platform	48.5 MHz frequency; 400 mV power	Human mesenchymal stem cells	Decreased cell attachment, improved metabolic activity	Cell structural stiffness	(*122*)
Low-intensity pulsed ultrasound	1 MHz frequency; 30–250 mW/cm^2 intensity; 600 ms pulse duration	Human mesenchymal stem cells	Chondrogenic differentiation	TNF signaling	(*123*)
Low-intensity continuous ultrasound	5 MHz frequency; 2.5 Vpp voltage	Human mesenchymal stem cells	Chondrogenic differentiation	Nuclear localization of SOX9, phosphorylation of ERK1/2, and disruption of actin filaments	(*124*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 2, 15, and 30 mW/cm^2 of intensities	Rat mesenchymal stem cells	Osteogenic differentiation	ERK1/2 and p38 intracellular signaling pathways	(*125*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Mouse mesenchymal stem cells	Suppressed adipogenesis and promoted osteogenesis	ROCK-Cot/Tpl2-MEK-ERK signaling pathway and the modulation of PPARγ2 activity	(*126*)
Low-intensity pulsed ultrasound	0.25 MHz frequency	Human mesenchymal stem cells	Proliferation	ERK1/2 and PI3K-Akt signaling pathways	(*136*)
Low-intensity pulsed ultrasound	1 MHz frequency; 250 and 750 mW/cm^2	Rat mesenchymal stem cells	Proliferation	MAPK pathway	(*137*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Human mesenchymal stem cells	Proliferation	MAPK/ERK and PI3K/AKT signaling pathways	(*138*)
Low-intensity pulsed ultrasound	0.25 MHz frequency; 30 mW/cm^2	Rat mesenchymal stem cells	Migration	FAK-ERK1/2 signaling pathway	(*139*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Rat mesenchymal stem cells	Cell-to-cell communication	ERK1/2 and p38 signaling pathways	(*140*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Rat mesenchymal stem cells	Cell recruitment	SDF-1/CXCR4 signaling pathway	(*215*)
Low-intensity pulsed ultrasound	47 MHz frequency; 112 mW/cm^2 intensity	Human mesenchymal stem cells	Modulation of Ca^{2+} signaling	Opening of connexin 43 hemichannels on the plasma membrane	(*216*)
Acoustics-induced vibration platform	30–80 nm vibration displacement and 1 kHz frequency	Human mesenchymal stem cells	Tenogenic differentiation	NF-κB signaling cascade	(*217*)
Low-intensity pulsed ultrasound	2 MHz frequency; 20 and 30 mW/cm^2 intensity	Human adipose stem cells	Osteogenic differentiation	Up-regulation of HSP 70, HSP90, and bone morphogenetic protein signaling pathway	(*142*)
Low-intensity pulsed ultrasound	1 MHz frequency; 5–10 V voltage	Rat neural stem cells	Proliferation and differentiation	Notch signaling pathway	(*141*)
Acoustics-induced vibration platform	~30 nm vibration displacement and 1 kHz frequency	Human mesenchymal stem cells	Osteogenic differentiation	Nanoscale mechanotransduction	(*72*)
Acoustics-induced vibration platform	~30 nm vibration displacement and 1 kHz frequency	Human mesenchymal stem cells	Osteogenic differentiation	Cholesterol sulfate–mediated stimulation	(*73*)

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(*Continued*)

Acoustic technologies	Parameters	Cell source	Cellular functions	Biological mechanisms	Refs.
Functionalized microbubble-based platform	1 MHz frequency; acoustic pressure amplitude of 0.025 or 0.08 MPa	Human mesenchymal stem cells	Osteogenic differentiation	Cytoskeletal contractility and YAP activation	(*69*)
Acoustics-induced vibration platform	90 nm vibration displacement and 1 kHz frequency	Human mesenchymal stem cells	Osteogenic differentiation and the induction of ROS and inflammation	Mechanosensitive ion channels	(*218*)
Orchestration of tissue progenitor cells					
Acoustics-induced vibration platform	40.6 nm vibration displacement and 1 kHz frequency	Human osteoclasts	Inhibition of osteoclastogenesis	Akt signaling pathway	(*213*)
Acoustics-induced vibration platform	100 Hz frequency	Human fibroblasts	Cell migration	Cell morphology and actin organization	(*145*)
Low-intensity pulsed ultrasound	0.5 MHz frequency; 150 mVpp voltage	Human epidermal keratinocytes	Cell proliferation and migration	PI3K/AKT and JNK signaling pathways	(*219*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Mouse osteosarcoma cells	Inhibition of proliferation and mitochondrial membrane potential	Akt, ERK1/2 signaling pathways	(*220*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Human fibroblasts	Proliferation	Activation of integrin receptors and a Rho/ROCK/Src/ERK signaling pathway	(*146*)
High-intensity continuous ultrasound	20 kHz frequency; 400 W/cm^2 intensity	Human fibroblasts	Cell proliferation and migration, increased production of the extracellular matrix	Activation of p38 and ERK1/2 MAPK signaling pathways	(*147*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Mouse osteoblasts	RANKL, MCP-1, and MIP-1β expression	Regulation of angiotensin II type 1 receptor	(*79*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Mouse osteoblasts	Inhibition of inflammatory responses of LPS-stimulated osteoblasts	Inhibition of TLR4/MyD88 complex formation	(*221*)
Orchestration of endothelial cells					
Low-intensity pulsed ultrasound	1 MHz frequency; 47.12 mW/cm^2 intensity	Human aortic endothelial cells	Prevention of the oxidative stress–induced endothelial-mesenchymal transition	Inhibition of ROS accumulation and activation of the PI3K/Akt signaling cascade. Inhibition of cell migration and excessive ECM deposition	(*154*)
Low-intensity continuous ultrasound	1 MHz frequency; 300 mW/cm^2 intensity	Human umbilical vein endothelial cells	Inhibition of apoptosis	Mitochondrial-dependent intrinsic apoptotic pathway	(*155*)
Low-intensity pulsed ultrasound	0.5 MHz frequency; 70–280 mW/cm^2 intensity	Human umbilical vein endothelial cells	Promotion of apoptosis and inhibition of angiogenesis	P38 signaling–mediated endoplasmic reticulum stress	(*156*)
Microbubble-aided ultrasound	1 MHz frequency; 0.1 MPa spatial negative peak pressure	Human umbilical vein endothelial cells	Increased intracellular H_2O_2 levels, protein nitrosylation, and a decreased endogenous glutathione level	Increased membrane permeability	(*162*)
Acoustic cavitation	1.25 MHz frequency; 0.24 MPa spatial negative peak pressure	Mouse endothelial cells	Generation of immediate Ca^{2+} changes in cells	Direct contact with microbubbles	(*163*)
Low-intensity continuous ultrasound	1 MHz frequency; 300 mW/cm^2 intensity	Human umbilical vein endothelial cells	Angiogenesis	PI3K-Akt-eNOS signaling pathway	(*222*)
Low-intensity pulsed ultrasound	1.5 MHz frequency; ~30 mW/cm^2 intensity	Human umbilical vein endothelial cells	Angiogenesis	YAP/TAZ signaling pathway	(*161*)
Orchestration of nerve cells					
Low-intensity pulsed ultrasound	500 kHz frequency; 0.3 MPa acoustic pressure	Mouse neurons	Neuron activation	Mechanosensitive ion channel Piezo1	(*168*)

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(*Continued*)

Acoustic technologies	Parameters	Cell source	Cellular functions	Biological mechanisms	Refs.
Dual-frequency low-intensity ultrasound	1138 and 560 kHz frequency; 40 kPa of peak pressure	Rat neural stem/ progenitor cells	Differentiation	Stable cavitation	(*171*)
Low-intensity, low-frequency ultrasound	500 kHz frequency; 11.52 kPa of spatial peak pressure	Mouse astrocytes	Neuromodulation	Opening of TRPA1 channels	(*169*)
Orchestration of immune cells					
Low-intensity pulsed ultrasound	1.5 MHz frequency; 30 mW/cm^2 intensity	Mouse macrophages	Phagocytosis	Actin polymerization, Rho, and Src/MAPKs signaling pathways	(*183*)
Acoustofluidics	96.7 MHz frequency; 3.65 Vpp voltage	Human NK-92 cell line	Enhanced immune activity	Calcium influx	(*214*)
Orchestration of cancer cells					
Acoustics-induced vibration platform	20 Hz frequency; 140 μm of maximum amplitude	Human epidermoid carcinoma cell	Apoptosis	Regulation of glucose consumption	(*184*)
Acoustic cavitation	1 MHz frequency	Human pancreatic cancer cell lines	Apoptosis	Activation of Piezo1	(*189*)

which provides a comprehensive summary of specific technologies, relevant acoustic parameters, affected cellular functions, and the underlying biological mechanisms.

Orchestration of stem cells via acoustics

Stem cells, particularly embryonic stem cells (ESCs) and MSCs, exhibit remarkable plasticity and responsiveness to mechanical and biochemical cues, which regulate self-renewal, differentiation, and lineage commitment (*111*–*114*). Unlike traditional biochemical differentiation factors, acoustic forces provide a noninvasive, tunable means of influencing stem cell fate through precise mechanotransduction mechanisms, making them particularly attractive for applications in regenerative medicine and tissue engineering.

Acoustic stimulation has been shown to activate integrin-mediated mechanotransduction, intracellular calcium signaling, cytoskeletal remodeling, and gene transcription of stem cells, all of which influence their differentiation and functional adaptation (*68*, *69*). The primary acoustic technologies investigated in stem cell research include SAWs, low-intensity pulsed ultrasound, and microbubble-integrin mechanotransduction (*68*, *115*, *116*). These techniques offer a highly controllable platform for modulating ESC and MSC behaviors in vitro, providing insight into how mechanical forces contribute to cellular development. This section will first discuss the influence of acoustic stimulation on ESC differentiation and MSC fate determination, followed by an exploration of other acoustic techniques and their effects on additional stem cell types.

ESC differentiation induced by acoustic stimulation

ESCs are pluripotent cells capable of differentiating into a wide range of specialized cell types under appropriate stimuli. The role of mechanical forces in early embryonic development has driven interest in acoustic stimulation as a regulatory tool for ESC differentiation. Among the most studied approaches is SAW-based stimulation, which applies substrate vibrations and acoustic streaming effects to induce controlled mechanical stress in ESC cultures. A SAW stimulation platform was developed by fabricating a rigid printed circuit board, prepatterned with a metal interdigitated electrode, which was mechanically clamped onto a $LiNbO_3$ wafer to produce SAWs. The SAWs propagate toward a polydimethylsiloxane cell culture chamber and interact with the cell culture medium to generate leaky SAWs. The leaky SAWs and the direct surface vibration of the $LiNbO_3$ substrate produce mechanical stimulations of the cultured ESCs, which induces great promotion of ESC differentiation toward neurons (Fig. 3A), as evidenced by Nestin and βIII-tubulin up-regulation (*115*).

Another promising approach for ESC differentiation is microbubble-integrin mechanotransduction, which enhances ESC-matrix interactions by functionalizing microbubbles with RGD peptides, allowing them to adhere to ESCs and act as mechanical force transmitters. This approach has been shown to down-regulate pluripotency transcription factors Oct4 and Nanog while increasing intracellular contractility and calcium activity (*68*, *117*). These mechanosensitive responses enhance ESC differentiation efficiency, particularly toward neuronal and mesodermal lineages, while also improving ESC survival and cloning efficiency by triggering adhesion-mediated survival signaling.

MSC fate determination and mechanotransduction

MSCs are highly mechanosensitive and play a crucial role in tissue regeneration, immune modulation, and musculoskeletal repair. Unlike ESCs, which require strict niche conditions to maintain pluripotency, MSCs respond dynamically to mechanical stimuli, ECM stiffness, and substrate topography to regulate their differentiation potential, proliferation, and migratory behavior (*118*–*121*). Acoustic stimulation has emerged as a powerful tool for guiding MSC lineage commitment, particularly toward osteogenic, chondrogenic, and myogenic fates, while also influencing MSC survival, proliferation, and paracrine signaling (*116*, *122*–*126*). Among the various acoustic technologies, low-intensity pulsed ultrasound has been the most widely studied for MSC differentiation, particularly in bone and cartilage tissue engineering. In addition, SAWs, integrin-bound microbubble stimulation, and acoustofluidic platforms have been investigated for their ability to modulate MSC behavior at the cellular and molecular levels.

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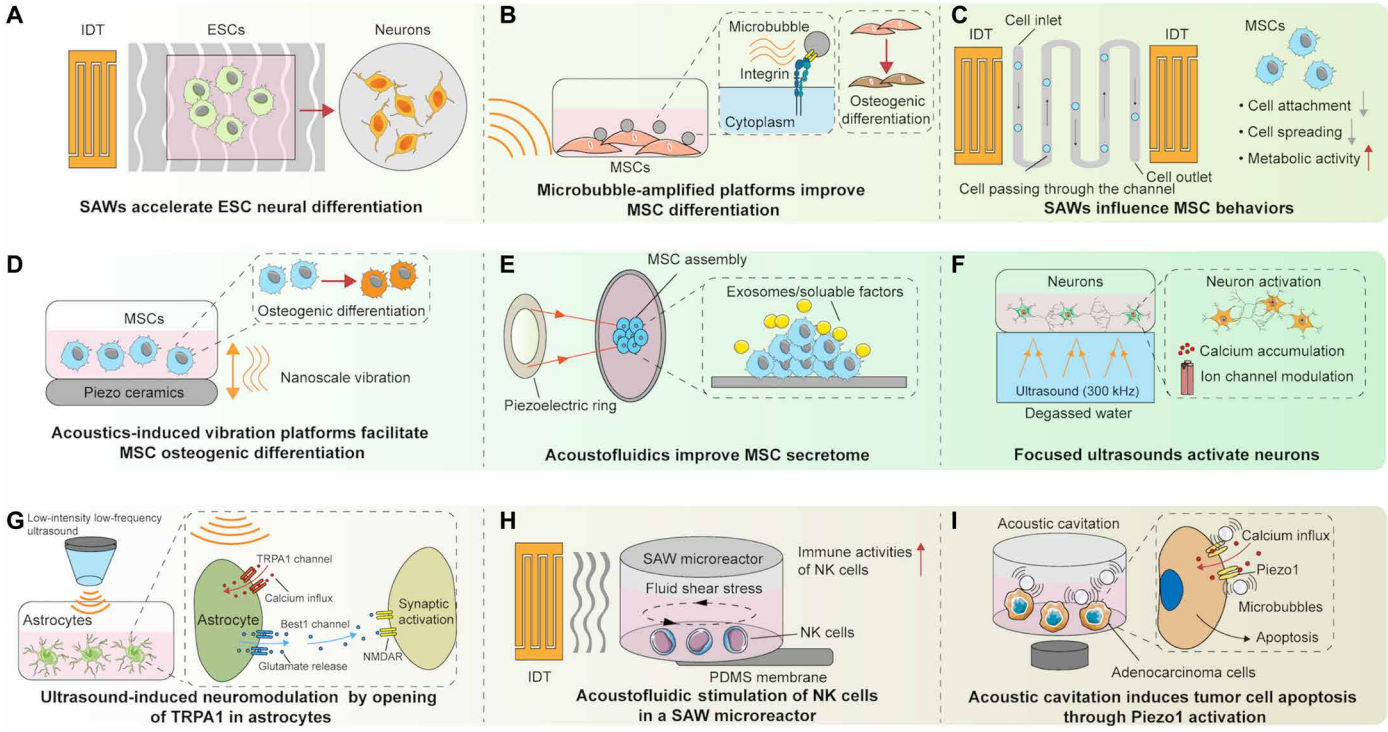


Fig. 3. Several acoustic platforms designed for orchestrating cellular functions. (**A**) A traveling surface acoustic wave platform stimulates embryonic stem cell (ESC) neural differentiation (*115*). (**B**) Functionalized microbubble-based platforms regulate stem cell differentiation (*68*, *69*). (**C**) A standing surface acoustic wave platform influences cell behaviors of MSCs, MG63, L929, and HaCaT cells (*122*). (**D**) Acoustics-induced vibration platforms facilitate MSC osteogenic differentiation (*72*, *73*). (**E**) An acoustofluidics platform enhances MSC secretome (*196*). (**F**) A focused ultrasound setup activates neuron through calcium accumulation and ion channel amplification (*89*). (**G**) A low-intensity, low-frequency ultrasound activates astrocytes through the opening of TRPA1 channels (*169*). (**H**) An acoustofluidics platform creates acoustic streaming flow in a SAW microreactor to apply shear stress to NK cells and improve their immune activities (*214*). (**I**) An acoustic cavitation platform induces pancreatic cancer cell apoptosis through Piezo1 activation (*189*). This figure was generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 4.0 unported license (https://creativecommons.org/licenses/by/4.0/deed.en) (Servier; https://smart.servier.com/).

Osteogenic differentiation of MSCs under acoustic stimulation. Osteogenic differentiation of MSCs is a key focus in bone tissue engineering and regenerative medicine, and low-intensity pulsed ultrasound has demonstrated great potential in directing MSCs toward an osteogenic lineage. Studies have shown that low-intensity pulsed ultrasound enhances MSC osteogenic differentiation by activating ERK1/2 and p38 MAPK signaling pathways, leading to increased expression of Runx2, ALP, and osteocalcin (*127*–*129*). These effects are observed across various intensities of low-intensity pulsed ultrasound (2, 15, and 30 mW/cm^2), with lower intensities demonstrating greater mineralization effects (*125*). Further mechanistic studies indicate that low-intensity pulsed ultrasound enhances calcium influx and cytoskeletal contractility, both of which contribute to osteogenic signaling. In addition, low-intensity pulsed ultrasound up-regulates integrin-ECM interactions, reinforcing the role of mechanotransduction in guiding MSC differentiation outcomes. Notably, low-intensity pulsed ultrasound-treated MSCs exhibit increased cell calcification and up-regulation of osteogenic markers such as Runx2 and osteocalcin mRNAs (*126*), supporting application in bone regeneration therapies.

Beyond low-intensity pulsed ultrasound, microbubble-based acoustic stimulation has been explored as an alternative osteogenic strategy. Studies using RGD-functionalized microbubbles have demonstrated that ultrasound-driven microbubble oscillations induce cytoskeletal contractility and activate the YAP pathway (Fig. 3B) (*69*). This method successfully induces osteogenic differentiation even when MSCs are cultured on soft substrates, which are typically inhibitory for osteogenesis. This suggests that acoustic forces can compensate for inadequate substrate stiffness, offering previously unidentified possibilities for bone regeneration in soft scaffolding environments.

Chondrogenic differentiation and cartilage regeneration. Chondrogenic differentiation of MSCs is another key application of acoustic stimulation, particularly for cartilage repair and osteoarthritis treatment. Studies have demonstrated that low-intensity pulsed ultrasound promotes MSC chondrogenesis by inhibiting autophagy and tumor necrosis factor (TNF) signaling (*116*, *123*) while activating the TGF-β1–integrin–mTOR pathway (*102*, *130*). In addition, SOX9, a master transcription factor for chondrogenesis, is up-regulated in low-intensity pulsed ultrasound-treated MSCs (*124*), leading to enhanced ECM deposition and proteoglycan synthesis. The effects of low-intensity pulsed ultrasound on MSC chondrogenesis appear to be intensity dependent, with specific intensities promoting either chondrogenic or osteogenic differentiation. This highlights the importance of precisely tuning acoustic parameters to achieve the desired differentiation outcome.

Adipogenic inhibition and MSC lineage commitment. While low-intensity pulsed ultrasound promotes osteogenic and chondrogenic differentiation, it has been reported to inhibit adipogenic differentiation in MSCs. Studies have shown that low-intensity pulsed ultrasound–treated

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MSCs exhibit impaired lipid droplet formation and reduced expression of adipogenic markers such as peroxisome proliferator-activated receptor gamma 2 (PPARγ2) and Fabp4 (*126*, *131*). Furthermore, low-intensity pulsed ultrasound down-regulates PPARγ2 phosphorylation and inhibits ERK signaling, effectively suppressing adipogenesis. Given that adipogenic differentiation typically occurs in soft matrix environments with low mechanical stress (*127*, *132*–*135*), it is reasonable that acoustic stimulation directs MSC fate away from adipogenesis toward osteogenic and chondrogenic lineages.

MSC proliferation, migration, and paracrine signaling under acoustic stimulation. Beyond differentiation, acoustic forces have been shown to enhance MSC proliferation, migration, and paracrine function, making them valuable for cell-based regenerative therapies (*136*–*139*). Studies indicate that low-intensity pulsed ultrasound promotes MSC proliferation by inducing cell cycle progression, particularly by shifting MSCs from G_0-G_1 to S and G_2-M phases (*136*). This effect is mediated by the up-regulation of CyclinD1 and c-Myc, two key regulators of cell cycle progression.

In addition, acoustic stimulation enhances MSC migration and recruitment, both of which are crucial for tissue regeneration. Low-intensity pulsed ultrasound has been shown to increase MSC migration speed (*139*), while also enhancing cell-cell communication and paracrine signaling (*128*, *140*). Notably, low-intensity pulsed ultrasound increases the secretion of growth factors, cytokines, and extracellular vesicles, suggesting that acoustic stimulation may improve MSC-based therapies by enhancing their regenerative secretome.

Alternative acoustic techniques for stem cell modulation

While low-intensity pulsed ultrasound, SAWs, and microbubble-integrin platforms have been extensively studied, several alternative acoustic techniques have also demonstrated potential for stem cell regulation. One such approach involves nanovibrational bioreactors, which generate nanoscale sinusoidal displacements (10 to 14 nm at 1 kHz) to enhance osteogenic differentiation through RhoA signaling (Fig. 3D) (*74*). In addition, bulk acoustic waves have been investigated for their ability to control stem cell patterning and assembly, particularly in three-dimensional scaffold systems (*129*). These findings suggest that acoustic modulation extends beyond differentiation control, potentially influencing cellular spatial organization and tissue structuring.

Acoustic effects on other stem cell types

In addition to ESCs and MSCs, acoustic stimulation has been investigated in neural stem cells (NSCs), adipose-derived stem cells (ASCs), hematopoietic stem cells (HSCs), and dental stem cells (DSCs). Low-intensity pulsed ultrasound has been reported to enhance NSC differentiation via Notch signaling (*141*), while acoustic cavitation has been shown to stimulate angiogenesis in ASCs by up-regulating HSP70 and HSP90 (*142*). Studies on HSCs have demonstrated that ultrasound can increase colony-forming potential (*143*), suggesting applications in hematopoietic transplantation. In addition, DSCs exposed to ultrasound stimulation exhibit enhanced proliferation and differentiation into odontogenic and osteogenic lineages, implicating ultrasound as a potential tool in dental tissue engineering (*144*).

Acoustic modulation of tissue progenitor cells

Tissue progenitor cells, including fibroblasts, myoblasts, and osteoblasts, play a pivotal role in wound healing, musculoskeletal regeneration, and ECM remodeling. Unlike stem cells, which have pluripotency or multipotency, progenitor cells have a more restricted differentiation potential but exhibit high mechanosensitivity, making them responsive to external acoustic stimuli. Given their role in tissue repair and regeneration, these cells have been widely investigated in response to low-intensity pulsed ultrasound, SAWs, and acoustic cavitation, which have been shown to regulate proliferation, differentiation, migration, and ECM synthesis.

This section explores how acoustic stimulation influences fibroblast activity, myoblast differentiation, and osteoblast function, followed by a discussion of alternative acoustic approaches and their impact on additional progenitor cell types.

Acoustic enhancement of fibroblast function and wound healing

Fibroblasts play a central role in tissue repair, ECM production, and inflammatory response. They secrete collagen, fibronectin, and cytokines that contribute to wound healing and tissue remodeling. Acoustic stimulation has been widely studied for its ability to enhance fibroblast proliferation, migration, and collagen synthesis, accelerating wound healing and scar formation (*145*–*147*).

Effects of low-intensity pulsed ultrasound on fibroblast proliferation and ECM synthesis. Reports of acoustic stimulation influencing fibroblasts date back to the 1980s (*148*). Ultrasound-induced cavitation has been shown to successfully improve collagen synthesis in fibroblasts. Subsequently, evidence emerged demonstrating that ultrasound (both 1 MHz pulsed ultrasound and 45 kHz continuous ultrasound) effectively promotes cell proliferation in fibroblasts (*149*). Low-intensity pulsed ultrasound has been shown to promote fibroblast proliferation and collagen production through ERK1/2 and TGF-β signaling (*146*).

Acoustic regulation of fibroblast migration and wound healing. Fibroblast migration is essential for tissue regeneration and scar formation. Studies indicate that low-frequency acoustic vibrations (~100 Hz) promote fibroblast motility and directional migration, while frequencies exceeding 100 Hz disrupt migration efficiency (*145*). Similarly, SAW-based microfluidic platforms have been used to guide fibroblast migration, demonstrating increased cell adhesion and directional motility in response to acoustic radiation forces (*150*). The cell migration speed is positively related to applied acoustic intensities. In addition, a short-term high-intensity ultrasound treatment (20 kHz, 400 W/cm^2) can also accelerate fibroblast proliferation and migration in vitro (*147*). According to RNA sequencing analysis, p38 and ERK1/2 MAPK pathway activation is responsible for the regulation of cell functions.

Osteoblast stimulation and bone regeneration under acoustic forces

As the main bone-forming cells, osteoblasts are responsible for mineralization and skeletal integrity. Given their dependence on mechanical cues, acoustic stimulation has been widely investigated for its ability to enhance osteogenic activity, ECM deposition, and mineralization. Low-intensity pulsed ultrasound has been shown to enhance osteoblast proliferation and differentiation through activation of RANKL, MCP-1, and MIP-1β, leading to increased Runx2, ALP, and osteocalcin expression (*79*, *151*). The effects of low-intensity pulsed ultrasound on osteogenesis are dose dependent, with low-intensity ultrasound (75 mW/cm^2) increasing osteoblast proliferation, while higher intensities (>400 mW/cm^2) reduce differentiation efficiency. SAWs have been explored as an alternative approach for osteoblast stimulation. Studies indicate that SAW exposure enhances ECM secretion, cell attachment, and mineralization (*152*). The primary mechanotransduction pathway involves YAP/transcriptional

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co-activator with PDZ-binding motif (TAZ) signaling, which regulates osteogenic gene expression in response to mechanical stress.

In comparison to stem cells, tissue progenitor cells generally exhibit a more singular and defined functionality. Therefore, the large-scale in vitro preparation of tissue progenitor cells with optimal therapeutic efficacy holds great promise in cell therapy. Various acoustic platforms have been demonstrated to effectively modulate the biological functions of tissue progenitor cells, with distinct parameters and configurations yielding differential effects. Consequently, future research directions should emphasize on the optimization of acoustic parameters and cell manipulation resolutions, thereby attaining optimal biological functionality for therapeutic applications.

Regulation of endothelial cells by acoustic technologies

Endothelial cells form the inner lining of blood vessels, serving as a critical interface between circulating blood and vascular tissues. These cells play a fundamental role in vascular homeostasis, angiogenesis, inflammation, and barrier function, dynamically responding to mechanical forces such as shear stress, cyclic strain, and ECM stiffness (*153*). Mechanotransduction in ECs is mediated by integrin signaling, ion channels, cytoskeletal remodeling, and nitric oxide production, allowing them to regulate vascular tone, permeability, and immune responses.

Acoustic stimulation has emerged as a promising noninvasive tool for modulating endothelial function, offering precise control over cell proliferation, migration, and angiogenesis (*154*–*156*). Unlike static mechanical stimuli, acoustic waves can penetrate deep into tissues, providing spatially controlled mechanical cues that mimic physiological forces. This section explores the effects of low-intensity pulsed ultrasound, SAWs, and acoustic cavitation on endothelial cells, followed by a discussion of alternative acoustic techniques and their effects on additional vascular cell types.

Acoustic enhancement of endothelial cell function

Endothelial cells respond to acoustic stimulation through mechanosensitive ion channels, cytoskeletal rearrangement, and nitric oxide signaling, all of which are essential for vascular integrity and function. Among various acoustic modalities, low-intensity pulsed ultrasound has been widely studied for its ability to enhance endothelial migration, angiogenesis, and permeability regulation.

Nitric oxide production and endothelial vasodilation. One of the most well-documented effects of low-intensity pulsed ultrasound on endothelial cells is its ability to enhance nitric oxide production, a key regulator of vascular tone and endothelial homeostasis. Studies have shown that low-intensity pulsed ultrasound up-regulates endothelial nitric oxide synthase (eNOS) activity, leading to increased nitric oxide synthesis, improved vasodilation, and reduced endothelial inflammation (*157*, *158*). The regulation of calcium influx via mechanosensitive ion channels also further promotes nitric oxide production and endothelial barrier function. These findings suggest that low-intensity pulsed ultrasound may serve as a therapeutic tool for treating vascular disorders, such as hypertension and atherosclerosis, where endothelial dysfunction and nitric oxide deficiency are contributing factors.

Endothelial migration and angiogenesis. Beyond nitric oxide production, acoustic stimulation has been shown to enhance endothelial migration and angiogenesis, both of which are critical for vascular repair and tissue regeneration. Studies indicate that low-intensity pulsed ultrasound promotes endothelial sprouting, capillary formation, and Flk-1 expression (*159*). Exposure to acoustic waves up-regulates VEGF-A, CD29, and SDF-1. They are all key regulators of angiogenic signaling and endothelial survival (*160*). Mechanistic studies suggest that phosphatidylinositol 3-kinase (PI3K)–Akt-eNOS and YAP/TAZ signaling (*161*) play key roles in acoustic-driven angiogenesis, highlighting the importance of mechanotransduction in vascular remodeling and wound healing.

Cavitation-induced endothelial activation. Acoustic cavitation, generated through ultrasound-driven microbubble oscillations, has also been explored as a tool for endothelial mechanostimulation. Studies indicate that cavitation-induced microstreaming effects enhance endothelial proliferation and migration, improving vascular regeneration without compromising cell viability (*162*, *163*). Unlike high-intensity ultrasound, which may induce thermal damage, acoustic cavitation provides a controlled mechanostimulation approach suitable for vascular engineering applications.

Acoustic modulation of smooth muscle cells and vascular remodeling

Vascular smooth muscle cells (VSMCs) are the primary regulators of vascular tone, arterial stiffness, and blood flow, dynamically responding to mechanical forces such as shear stress, cyclic strain, and ECM stiffness. Dysregulated VSMC behavior contributes to hypertension, atherosclerosis, and restenosis, making them a key target for acoustic-based vascular therapy. Studies indicate that low-intensity pulsed ultrasound modulates VSMC phenotype switching, a process essential for maintaining vascular homeostasis. Research has shown that low-intensity pulsed ultrasound down-regulates miR-17-5p while up-regulating PPAR-γ, two critical regulators of VSMC contractility and plasticity (*164*). In addition, SAWs influence calcium signaling and cytoskeletal tension in VSMCs, leading to improved vascular compliance and reduced arterial stiffness (*150*). These findings suggest that acoustic stimulation could serve as a noninvasive tool for treating vascular diseases by restoring VSMC function and preventing pathological remodeling.

Neuromodulation by acoustic technologies

Acoustic stimulation influences brain physiology and neuronal activity, leading to various neuromodulatory effects such as alterations in neuron metabolism, neural firing patterns, synaptic functions, and neurotransmitter release. Numerous in vivo tests have been performed to apply acoustic waves to targeted brain regions and provide precise spatial and temporal control over neural excitation or inhibition (*82*, *165*, *166*). In vitro applications of ultrasound have also successfully enhanced neuronal excitability (*167*). Acoustic stimulation directly influences membrane potential, calcium influx, and neurotransmitter release, leading to changes in neuronal excitability and network activity.

Acoustic stimulation of neuronal activity

Concurrently, substantial progress has been made in investigating how neurons react to acoustic waves. One example is the use of focused ultrasound to target and treat cortical neurons. The ultrasound induces an influx of calcium through the mechanosensitive ion channel TRPP1/2 complex and TRPC1 mechanoreceptors (Fig. 3F) (*89*). Subsequently, calcium-gated sodium channels amplify this signal, resulting in a strong spike in activity. The ultrasound treatment exerts a direct mechanical influence on cells independent of temperature change, acoustic cavitation, or a large deformation of cell morphology. In another study, a low-intensity, low-frequency ultrasound treatment also successfully induced calcium influx and signaling, primarily through Piezo1, as evidenced by a marked reduction

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in neuronal activation by ultrasound upon Piezo1 inhibition (*168*). In addition, the expression of several neuron activation-related proteins, such as phospho-CaMKII, phospho-CREB, and c-Fos, was increased following ultrasound treatment; however, this promotion effect can be reduced after inhibiting Piezo1's activity. Thus, different mechanosensitive ion channels have varying sensitivities to different acoustic stimulation patterns, but they will ultimately lead to an influx of calcium and neuron activation.

Acoustic modulation of glial cells and neuroprotection

Beyond neurons, glial cells play a crucial role in neuroinflammation, synaptic regulation, and tissue repair. Acoustic stimulation has been shown to modulate astrocyte and microglial function, suggesting that mechanical forces could be leveraged for neuroprotection and neuroregeneration. Low-intensity pulsed ultrasound can activate astrocytes through the opening of TRPA1 channels and the calcium influx induces the release of glutamate through Best1 channels in astrocytes (Fig. 3G) (*169*). In addition, low-intensity pulsed ultrasound enhances astrocytic secretion of brain-derived neurotrophic factor, a neurotrophic factor essential for neuronal survival, synaptic plasticity, and axonal outgrowth, through the activation of TrkB-Akt and calcium-CaMK signaling pathways (*170*). Moreover, the establishment of a stable acoustic cavitation can promote neurite outgrowth and the differentiation of neural stem/progenitor cells (*171*). These findings indicate that acoustic stimulation could be used to promote neural recovery following ischemic stroke, traumatic brain injury, and neurodegenerative disease.

Acoustic stimulation has also been investigated for its role in peripheral nerve regeneration and myelination. Research indicates that low-intensity pulsed ultrasound enhances Schwann cell proliferation and myelin production by up-regulating ErbB3, neuregulin-1, and Egr2/Krox20 (*172*). These findings highlight the potential of acoustics-based therapies for promoting functional recovery in spinal cord injuries and demyelinating diseases.

Immunomodulation by acoustic technologies

The discovery of immunomodulation is a great advancement in many areas of modern medicine, particularly for developing highly effective antitumor and antibacterial therapies (*173*, *174*). Plasticity is an essential property for various immune cells, such as macrophages and dendritic cells (*175*, *176*). The plasticity of immune cells, which refers to their capacity to switch their phenotype and function in response to different microenvironmental cues, is integral to maintaining immune homeostasis and orchestrating appropriate immune responses. Because most immune cells exhibit mechanosensitivity, acoustic stimulation emerges as a promising avenue to modulate immune cell plasticity and consequently refine immunomodulatory processes. Many in vivo acoustic treatments have been performed and demonstrated substantial efficacy in regulating in vivo immunomodulation. For example, targeted ultrasound stimulation applied directly to the spleen has been shown to reduce the severity of arthritis (*177*). However, the in vitro regulation of immune cell functions using acoustics remains in its early stages. While macrophages are the most extensively studied cell type, adaptive immune cells, such as T and B cells, have received comparatively less attention. Future research should prioritize these cell types to deepen our understanding of their acoustic modulation.

Acoustic stimulation of macrophage activation and polarization

Macrophages are central regulators of innate immunity and inflammation, displaying remarkable plasticity in their ability to adopt pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes. M1 macrophages produce TNF-α, interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS), driving immune responses against pathogens and tumors, whereas M2 macrophages secrete IL-10 and TGF-β, facilitating tissue repair and immune suppression. Acoustic stimulation has been shown to directly influence macrophage polarization in vitro. Current studies have reported the phenotype switch and immunomodulation effects of macrophages after being treated by low-intensity pulsed ultrasound in vitro (*178*–*181*). A study has identified the optimal stimulation conditions for low-intensity pulsed ultrasound on M1 macrophages to mitigate their pro-inflammatory effects (*182*). The recommended parameters are a frequency of 38 kHz, an intensity of 250 mW/cm^2, and a treatment duration of 90 min.

Beyond polarization, acoustic stimulation has been shown to enhance macrophage phagocytosis, a key process in immune defense and tissue homeostasis. SAW-induced oscillations stimulate actin polymerization and activate the Rho-Src/MAPK pathway, leading to increased engulfment of bacterial pathogens and apoptotic cells (*183*). This finding suggests that acoustic forces could be applied to improve macrophage-mediated clearance of infections, debris, and damaged tissue, offering previously unidentified possibilities for treating chronic inflammatory diseases and tissue repair therapies.

Regulating cancer cell behavior and tumor therapy by acoustic technologies

Typically, HIFU is used to induce cancer cell death by producing local hyperthermia and destructive cavitation. However, for practical applications, the utilization of HIFU will inevitably damage healthy tissues. In addition, acoustic sonoporation presents an alternative modality for tumor targeting, facilitating the localized administration of chemotherapeutic agents or genetic material. Nevertheless, researchers using this method should also pay more attention to the potential side effects on healthy cells and tissues. Therefore, developing gentle acoustic treatments that can selectively inhibit cancer cell functions without affecting normal cells is critical (*184*, *185*). Recently, low-intensity pulsed ultrasound has been reported to achieve selective ablation of various cancer cell types, including breast, colon, and leukemia cancer cells (*186*). The contrasting biomechanical characteristics between cancer cells and healthy cells, such as cytoskeletal stiffness, DNA content, nuclear-nucleolar volume ratios, and other viscoelastic properties, give rise to distinct responses of cancer cells to ultrasounds with specific parameters when compared to healthy cells. After a comprehensive screening, low-intensity pulsed ultrasound, applied at frequencies ranging from 0.5 to 0.67 MHz and a pulse duration of more than 20 ms, was found to selectively disrupt various cancer cell types—including breast, colon, and leukemia cells—while sparing healthy immune and red blood cells. Another study confirmed that, unlike HIFU, which causes direct structural damage, the utilization of a proper dosage of low-intensity ultrasound treatment can induce cancer cell apoptosis via regulating the Ca^{2+}/mitochondrial pathway (*187*). The sonoporation method is also reported to exhibit distinct cell survival efficiency toward different cancer cell types (*188*). The different combinations of the major parameters of sonoporation treatment, including microbubble concentrations, sound intensities, and irradiation time, show statistically different cell survival efficiencies. Another study shows that microbubble-based acoustic sonoporation can successfully induce pancreatic cancer cell apoptosis through activation of the mechanosensitive channel Piezo1 (Fig. 3I) (*189*). The primary challenge in

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using acoustic technologies to target tumor cells lies in achieving selectivity—specifically, how to effectively damage tumor cells while ensuring the safety of surrounding healthy somatic cells. This issue becomes even more critical in in vivo experiments, where the complex physiological microenvironment includes diverse cell types and noncellular components. As such, the focus of future research should be on developing advanced acoustic technologies with enhanced selectivity, with the ultimate goal of translating these innovations into effective in vivo therapeutic applications.

SUMMARY AND OUTLOOK

Therapeutic ultrasound has a long-standing history of clinical applications. Despite ultrasound's impressive outcomes in areas such as wound healing, pain relief, and targeted drug delivery, the full potential of acoustic-based therapies remains constrained by an incomplete understanding of how different ultrasound modalities influence cellular functions (*190*–*192*). It is well established that mechanical stimuli play a fundamental role in embryogenesis, tissue regeneration, and immune responses, largely owing to the widespread presence of mechanosensitive cells throughout the human body. Acoustic technologies provide an ideal platform to harness cellular mechanosensitivity, offering previously unidentified avenues for noninvasive therapeutic interventions. However, two primary limitations hinder their widespread application in both in vitro studies and in vivo treatments. First, the resolution of current in vitro acoustic platforms remains insufficient for detailed cellular analysis. Achieving single-cell or subcellular-scale stimulation is technically challenging, making it difficult to precisely investigate biomechanical mechanisms at the molecular level. Second, selective, localized control of acoustic stimulation in vivo remains a challenge. The development of portable, high-precision acoustic systems capable of delivering targeted mechanical forces to specific tissues or cell populations is crucial for translating laboratory findings into clinical applications.

Advancing acoustic precision: Identifying effective acoustic parameters

One of the most pressing needs in the field is the establishment of standardized acoustic parameters for different cell types and biological effects, addressing concerns regarding variability and reproducibility. Extensive studies have demonstrated that the biological effects of acoustic stimulation are highly dependent on frequency, intensity, and exposure duration, with distinct parameters yielding differential cellular responses across mechanosensitive cells. For example, as mentioned in the last section, low-intensity pulsed ultrasound has been widely studied for its ability to regulate stem cell behavior, with specific intensities and duty cycles promoting either MSC proliferation or lineage-specific differentiation. Studies indicate that low-intensity pulsed ultrasound at 30 mW/cm^2 enhances MSC osteogenic differentiation through ERK1/2 and p38 signaling, whereas lower intensities, such as 15 mW/cm^2, support chondrogenic differentiation by activating TGF-β1–integrin–mTOR pathways (*125*, *126*). In contrast, intensities exceeding 50 mW/cm^2 may lead to reduced viability or unintended differentiation outcomes, highlighting the importance of precise parameter optimization. Similarly, in neuromodulation, focused ultrasound can enhance neuronal excitability at frequencies between 250 kHz and 1 MHz, inducing calcium influx through TRPP1/2 and Piezo1 channels (*89*, *168*). However, higher-intensity stimulation in the same range can inhibit synaptic activity, demonstrating bidirectional control over neural circuits depending on acoustic intensity. This has profound implications for neurological disorder treatment, where both excitatory and inhibitory neuromodulation may be required for conditions such as epilepsy, depression, or Parkinson's disease (*167*, *170*).

To position acoustic technologies at the forefront of cell biology and clinical applications—particularly in the study of cellular mechanobiology and the development of therapeutic tools—it is crucial to gain a clearer understanding of the dose-dependent effects (e.g., frequencies, intensities, and durations) of acoustic stimulation on various cell types. Achieving this requires overcoming two key challenges. First, the creation of widely applicable, reproducible, and mechanistically well-defined acoustic stimulation platforms. These platforms must elucidate the specific types of acoustic forces generated and their effects on cells, distinguishing between direct mechanical wave stimulation and indirect force transmission via the ECM-cytoskeleton coupling. The second challenge involves conducting comprehensive response assessments across diverse cell types. A practical starting point is highly mechanosensitive cells, such as stem cells, where systematic studies can be performed across varying frequencies and intensities. By integrating omics-based approaches with molecular biology techniques, researchers can analyze gene expression changes and cellular functional adaptations triggered by acoustic stimulation, ultimately advancing our understanding of mechanotransduction mechanisms and enabling more precise translational applications.

Expanding acoustic applications: Acoustofluidics, biomaterials, sonogenetics, and micro-robots

One of the most promising advancements in acoustic technologies is the development of on-chip acoustofluidic platforms, which integrate acoustics with microfluidic systems to achieve high-resolution manipulation of cells, extracellular vesicles, and biomolecules (*193*–*198*). Acoustofluidic devices have been successfully used to direct stem cell differentiation and enhance paracrine signaling (*129*, *196*). More recently, the HANDS platform (harmonic acoustics for a noncontact, dynamic, selective particle manipulation) has demonstrated precise control over single-cell motion (*197*, *198*), reaffirming the great potential of acoustofluidics for high-resolution cell studies and therapeutic applications. However, further improvements are needed to increase efficiency across diverse cell types, particularly for enabling in vivo acoustic applications.

Beyond acoustofluidics, the integration of acoustic technologies with biomaterials, genetic engineering, and tissue engineering represents a key area of future development (Fig. 4). Piezoelectric biomaterials, which generate electrical signals in response to ultrasound, provide a unique platform for modulating cellular functions remotely (*199*–*201*). These materials have been fabricated into biocompatible scaffolds, coatings, and nanoparticles, demonstrating potential applications in tissue regeneration, antibacterial therapy, and drug delivery (*201*–*204*).

Similarly, sonogenetics has emerged as a highly promising strategy for combining acoustics with molecular biology, enabling remote, noninvasive control of genetically engineered cells (*205*, *206*). This technique uses ultrasound-responsive mechanosensitive ion channels, such as TRPV1, TRP-4, and MscL, to regulate cellular functions (*207*). Recent studies suggest that ultrasound-based activation of CAR-T cells using microbubble-assisted low-intensity ultrasound enhances cancer immunotherapy efficacy (*33*, *206*), marking a critical step toward precision-controlled cellular therapies.

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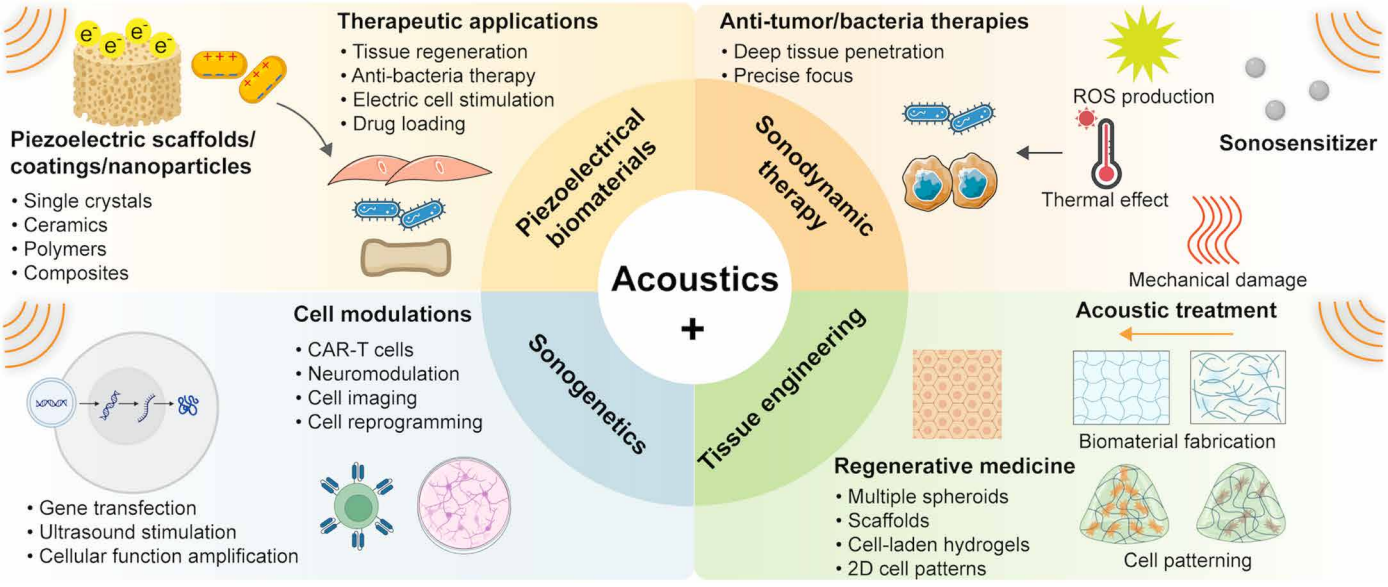


Fig. 4. The integration of acoustic technologies with other biomedical tools. Illustration showing that acoustics lies at the intersection of various promising applications involving piezoelectric biomaterials, sonodynamic therapy, sonogenetics, and tissue engineering. This figure was generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 4.0 unported license (https://creativecommons.org/licenses/by/4.0/deed.en) (Servier; https://smart.servier.com/). Created in BioRender. Ma, Z. (2025); https://BioRender.com/gmnx56i.

An emerging and highly promising direction in in vivo biomedical applications is the use of ultrasound micro-robots for precision therapy (*208*–*210*). These ultrasound-powered micro- and nano-robots are designed to navigate biological environments with high precision, offering unprecedented opportunities for targeted drug delivery, tissue repair, and immune cell modulation. Research has demonstrated that acoustic waves can remotely control the motion and behavior of micro-robots, allowing them to perform complex biomedical tasks such as transporting therapeutic agents and controlling objects in vivo (*208*, *211*, *212*). By leveraging ultrasound's deep tissue penetration and spatiotemporal precision, these micro-robots hold immense potential for next-generation, minimally invasive therapies. However, challenges remain in optimizing biocompatibility, real-time imaging capabilities, and precise steering within complex physiological environments. Future research should focus on developing intelligent, bio-hybrid micro-robots capable of responding dynamically to physiological cues, further bridging the gap between mechanical stimulation and biological function.

Perspectives

This review highlights the diverse and rapidly evolving landscape of acoustic technologies for cellular modulation and therapeutic applications. Although many advances remain in their early stages, ongoing innovation in acoustic platforms, mechanobiology, and bioengineering will likely drive the broader adoption of acoustics-based tools in biomedical research and clinical settings. As these technologies continue to advance, it is essential to improve their precision, reproducibility, and effectiveness across different cell types and biological processes.

By establishing optimized acoustic parameters, enhancing resolution in acoustic manipulation, and developing more sophisticated bio-integrated systems, researchers will gain deeper insights into how mechanical forces regulate cellular function. Efforts must be made to bridge the gap between in vitro studies and real-world medical applications, ultimately enabling the translation of acoustic-based approaches into effective, noninvasive therapies for tissue regeneration, immunotherapy, neuromodulation, and cancer treatment.

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