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REVIEW

Advanced Strategies for Ultrasound Control and Applications in Sonogenetics and Gas Vesicle-Based Technologies: A Review

Jinpeng Du[1,*], Min Liao[2,*], Daimo Zhang[3], Xiangnan Li[1]

[1]Department of Thoracic Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, People's Republic of China; [2]Department of Ultrasound, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China; [3]Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jinpeng Du; Xiangnan Li, Email dujinpenghust@163.com; lxn-2000@163.com

Abstract: Control systems play an important role in the diagnosis and treatment of medicine. In contrast to light and magnetic fields, ultrasound has received much attention due to its non-invasive, cost-effective, convenient, and high spatiotemporal precision and deep-penetration characteristics. Some studies have developed special nanomaterials for therapy by controlling the production of reactive oxygen species through ultrasound irradiation. However, the complex functionalities and toxicity issues associated with these nanomaterials limit the development of ultrasound control systems. To overcome these challenges, ultrasound control systems based on synthetic biology have been developed, especially for sonogenetics and gas vesicles. The tunable thermal and mechanical effects of ultrasound act as the main triggering source, enabling engineered cells to perform sono-thermal or sono-mechanical genetic modifications in the targeted tissue. Based on an in-depth understanding of the relationship between ultrasound effects and the design, composition, and applications of engineered cellular technologies, in this review, we focus on recent activation strategies of ultrasound for sonogenetics and gas vesicles, including sono-thermal promoter switch, sono-thermal transient receptor potential channel, sono-mechanical activation and gas vesicles. In addition, applications of these advanced ultrasound control systems for cancer therapy, neural activity, visual recovery and functional imaging are presented. Finally, we discuss the current challenges faced and provide an outlook on the future developments in this evolving field.

Keywords: ultrasound control system, sono-thermal switch, sono-mechanical activation, sonogenetics, gas vesicles

1. Introduction

Control systems often play an important role in the field of medicine,[1–3] as they enable precise regulation and monitoring of various processes, thereby enhancing the accuracy and efficiency of treatments. Depending on the triggering form, control systems can be categorized as internal trigger, such as the specific physicochemical properties of tumor microenvironments,[4] and externally triggered systems, such as light, magnetic, electric fields and ultrasound.[5–7] Among the external control systems, in contrast to light, which exhibits limited penetration into deep tissues and magnetic fields, which are challenging to manipulate with precision and electric fields, which are associated with low biosafety and significant side effects,[8–10] ultrasound has received much attention due to its non-invasive, cost-effective, convenient, and high spatiotemporal precision and deep-penetration characteristics.[11–16]

The development of ultrasound control systems in medicine has been a popular research topic in recent years.[17,18] Some researchers have prepared acoustic sensitizers, such as organic sonosensitizers hematoporphyrin,[19,20] inorganic sonosensitizers TiO_2,[21–23] and metal-organic frameworks,[24,25] to increase the generation of reactive oxygen species by ultrasound-controlled irradiation, for cancer cells apoptosis and necrosis.[26]

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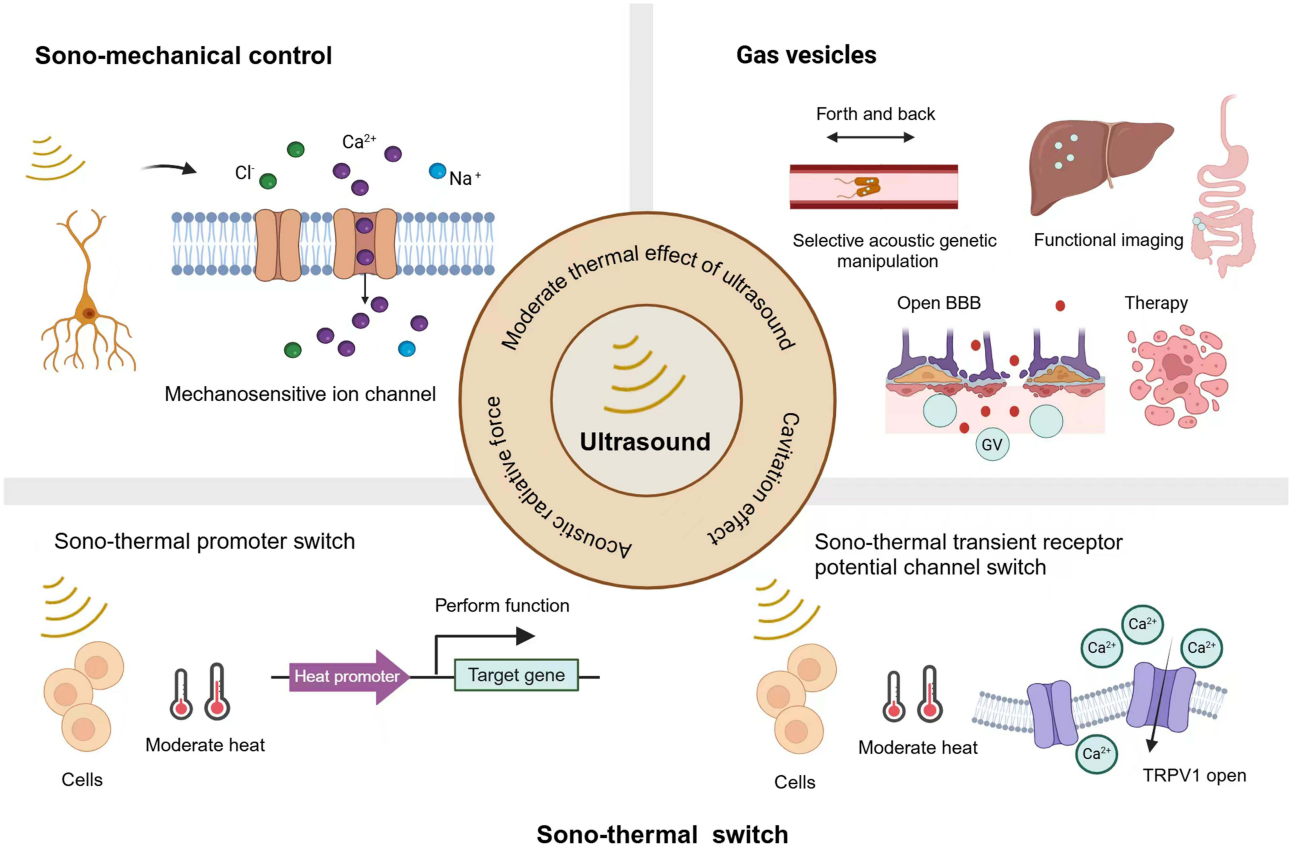
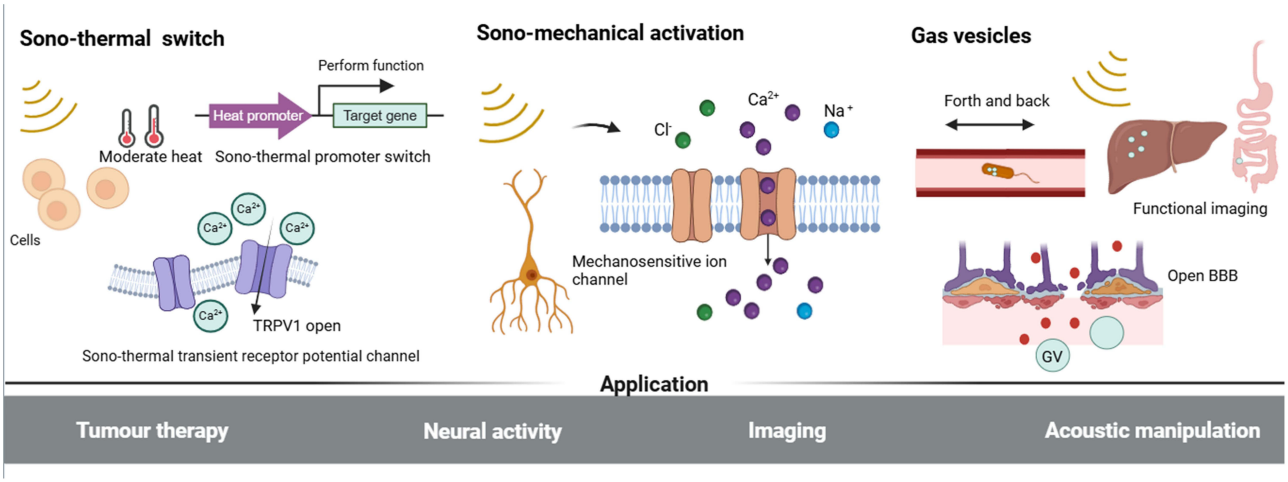
Graphical Abstract



However, the complex functionalities and toxicity issues of nanomaterials have been a concern, which limit the development and applications of the ultrasound control systems. Over the last decade, researchers have developed many tools to improve and optimize ultrasound control systems, notably through the application of synthetic biology, which has inspired many concepts for ultrasound activation systems. Synthetic biology emphasizes the engineering biomolecular systems and cellular functionalities,[27] including complex circuits and constructs. This approach has been applied in vaccine development,[28] microbiome engineering,[29] cell therapy,[30,31] as well as in the treatment of infectious diseases[32,33] and cancers.[34] The integration of synthetic biology with ultrasound-activated systems focuses on sonogenetics and gas vesicles. The tunable thermal and mechanical effects of ultrasound serve as the main triggering source, which allows engineered cells to exert sono-thermal genetics or sono-mechanical genetics in deep target tissue or the brain, as well as facilitating functional imaging and treatment of gas vesicle. However, few studies have offered a comprehensive overview of recent advances in activation strategies and application of ultrasound in sonogenetics and gas vesicles.

In this review, we summarize the activation strategies and application of ultrasound in sonogenetics and gas vesicle (Scheme 1). First, we discuss the moderate thermal effects, acoustic radiative forces and cavitation mechanisms associated with ultrasound activation in this field. Next, we provide a detailed control design of ultrasound in sonogenetics, including sono-thermal promoter switch, sono-thermal sensitive receptor potential channel activation and sono-mechanical activation, followed by a discussion about their biological applications in cancer treatment and neural activity, as well as the functional imaging, therapy and manipulation of gas vesicles. Finally, we conclude with an outlook on this research field, offering an in-depth discussion of the challenges and future developments. We believe this timely review will open new horizons for further exploration in the field of ultrasound activation system of medicine.



Scheme 1 The advanced control strategies and applications of ultrasound in medicine. Created in BioRender. Du, J. (2025) https://BioRender.com/yca5y1r.

2. Mechanisms of Ultrasound Activation

Ultrasound, as an external triggering tool, exhibits superior spatiotemporal and high-precision control ability in the manipulation fields.[7] Therefore, it holds a distinct advantage in the realm of control applications. Based on whether the temperature rises significantly, the effects of ultrasound in synthetic biology can be categorized into mild thermal and non-thermal effects. The non-thermal effects include cavitation effect and acoustic radiation force (ARF), both of which occur without a significant temperature elevation (Figure 1).

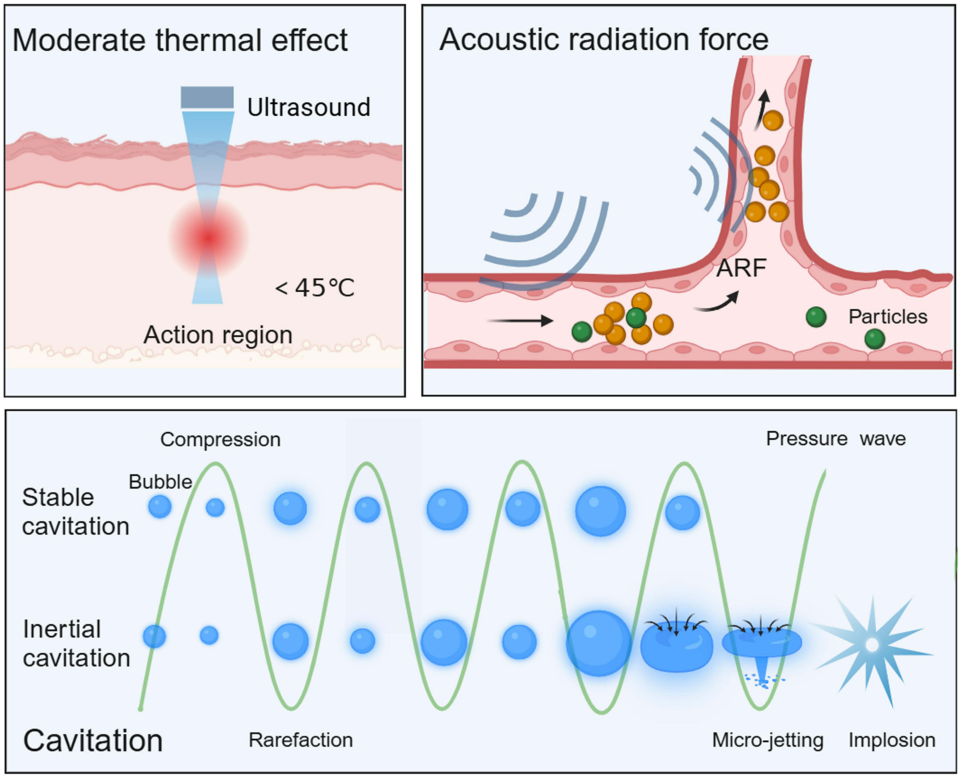


Figure 1 The mechanisms of ultrasound control in sonogenetics and gas vesicles, including moderate thermal effect, cavitation effect and acoustic radiation force.[35] Copyright 2020, American Chemical Society. Created in BioRender. Du, J. (2025) https://BioRender.com/f38slzv.

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2.1 Moderate Thermal Effect of Ultrasound

Ultrasound waves are mechanical waves. As they propagate through a medium, their energy is absorbed by the medium. The molecules of the medium vibrate in response to the ultrasound waves, generating friction among themselves. This friction converts the energy of the ultrasound waves into heat energy, thereby gradually reducing the acoustic intensity.[36,37] Different from the high-intensity focused ultrasound, which utilizes continuous waves with higher acoustic intensity to produce temperatures above 55°C for tissue coagulative necrosis,[38,39] ultrasound in synthetic biology employs a lower peak negative pressure to preserve cellular integrity. In addition, in order to prevent cell damage from thermal effects, a temperature slightly higher than the human body temperature, around 43°C, is chosen for use, referred to as moderate thermal effect. The controllability and flexibility of ultrasound parameters allow for this moderate temperature to be regulated by adjusting the duty cycle and action time. (Table 1 demonstrates the parameters of ultrasound used in synthetic biology).

2.2 Acoustic Radiation Force (ARF)

ARF is a physical phenomenon that occurs when an acoustic wave encounters an obstacle along its path.[45] The acoustic energy can be converted into mechanical momentum. ARF primarily affects cell activity in two ways: the mechanical activation of stress-sensitive ion gates and channels and the alteration of membranous potential and capacitance,[46,47] offering an opportunity for sonogenetics. In addition, ARF can also manipulate particles by controlling and designing acoustic field,[48–50] including levitation, movement, trapping, and even inducing the formation of particle clusters, as well as attracting or repelling particles.

2.3 Cavitation Effect

Cavitation effect is another non-thermal effect of ultrasound. With ultrasound irradiation, bubbles can exhibit two types of cavitation dynamics. Depending on whether the bubbles collapse or not, cavitation has two forms: stable cavitation and inertial cavitation. The pores of stable cavitation are smaller in cells (in the range of tens of nanometers to hundreds of nanometers), while the pores of inertial cavitation are larger (in the range of hundreds of nanometers to micrometers). These pores facilitate the uptake of loads with varying molecular weights.[51–53]

In the following section, we discuss the design and application of ultrasound activation for imaging and therapy, including the sono-thermal/mechanical switch in sonogenetics, and engineering gas vesicles.

3. Sono-Thermal Switch

Temperature serves as a distinctive input signal for sensing and responding to host conditions or specific external triggers like focused ultrasound.[43] An ideal bio-switch should exhibit a sharp thermal transition that leads to a significant change in activity, with the ability to adjust its switching temperature for a wide range of applications. Ultrasound has flexibility parameters that allow for the manipulation of local temperature, surpassing the limitations of optical approaches, which is typically limited to millimeters.

Moderate thermal ultrasound is often used in conjunction with a heat-inducible promoter, such as heat shock protein (HSP70), or disinhibition of the TcI, to trigger gene expression. Additionally, it can activate heat-sensitive transient receptor potential channels to alter motor behavior (Figure 2).

Table 1 Ultrasound Parameters Using Moderate Thermal Effect in Synthetic Biology

Frequency	Waves	Controlled Temperature in vivo	Action Time in vivo	Ref.
1.5 MHz	Pulsed waves	43 °C	15 min	[40]
1.5 MHz	Pulsed waves with 50% duty cycle	43 °C	Three pulses of 5 min	[41]
1.1 MHz	Pulsed waves	43 °C	10 min	[42]
1.5 MHz	Pulsed waves	41 °C	45 min-60 min	[43]
0.67 MHz	Pulsed waves with 0.6–0.7 MPa	37–43 °C	Between the temperatures of 43°C and 37°C every 5 min for 1 h	[44]

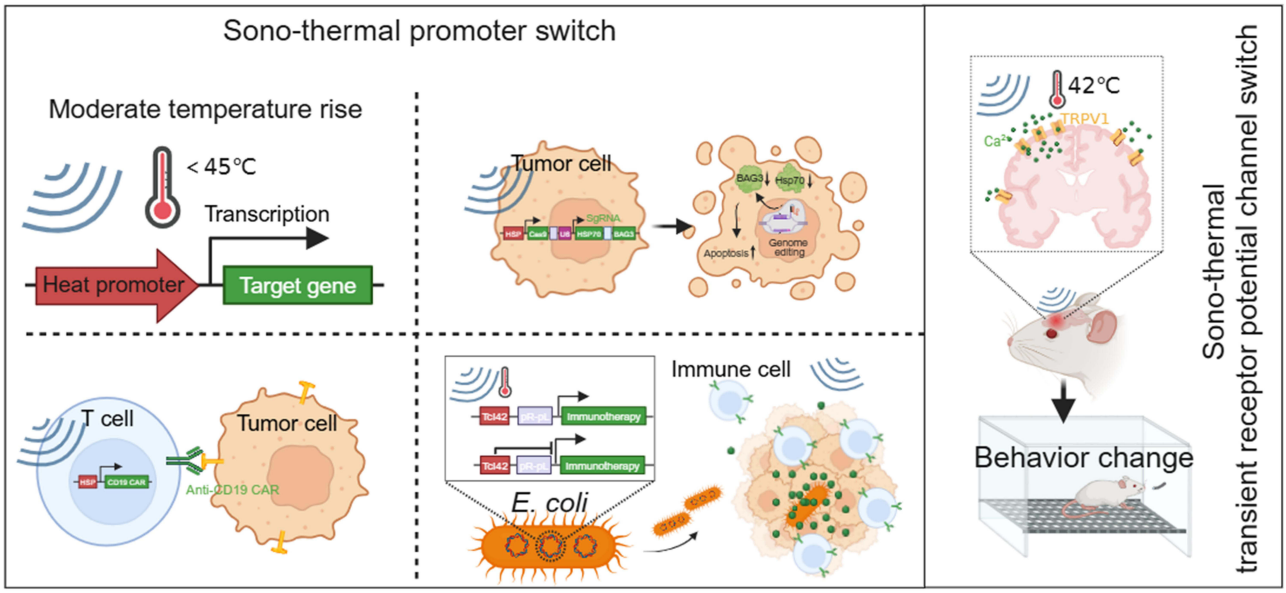

Figure 2 The design and application of sono-thermal promoter switch and sono-thermal transient receptor potential channel activation in medicine. Created in BioRender. Du, J. (2025) https://BioRender.com/32xkprw.

3.1 Sono-Thermal Promoter Switch

As a heat-inducible promoter, HSP70 can be activated by the mild local heating by focused ultrasound (FUS), thus enabling the spatiotemporal regulation of transgene expression. It was found that exposure to FUS at 43 °C for 2 minutes, the promoter activity increases at least 10-fold,[54] and shows a dose-dependent trend when local temperatures exceeded 42 °C. However, prolonged exposure at 45 °C led to decreased cell viability and a subsequent decrease in promoter activity. Additionally, repetitive heating at the same location could potentially sustain gene activation over prolonged periods. This method, which involves manipulating temperature, duration, and location, offers a direct, noninvasive means of spatially controlling gene activation.

FUS thermal stimulation was combined with chimeric antigen receptor (CAR)-T cell therapy to create a novel approach. Inducible CAR-T cells were engineered to be acoustogenetically and directly controlled by FUS without any external cofactors. By using the HSP promoter and heating at 43°C for 15 minutes, efficient activation of the reporter eGFP was observed (Figure 3A-D). To ensure sustainable gene activation and cellular functions for therapeutic purposes, the Cre-lox gene switch was integrated into this system (Figure 3E). The heat-induced CAR expression remained stable even 6 days after heat stimulation (Figure 3F). Through local temperature control in vivo using MRI-guided FUS, significant expression of the dual luciferase reporter gene was observed in mice. In addition, the growth of Nalm-6 and PC3 tumors was significantly suppressed after three pulses of 5-minute FUS stimulation at 43°C, demonstrating the efficacy of FUS-based sonogenetics in controlling CAR-T cells for the treatment of various types of tumors in vivo. This innovative FUS-based approach allows for the targeting of less optimal antigens without causing nonspecific off-tumor toxicity, thereby enabling precise control of CAR-T cells at specific sites.[41]

The heat-inducible CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) system was also investigated for tumor therapy, where researchers developed a nanodevice containing a heat-shock promoter-driven Cas9 plasmid, with single-guide RNA targeting HSP70 and BAG3. Upon localized mild thermal effect induced by FUS was applied to the targeted tumor tissue, the heat-inducible Cas9 plasmid is activated to initiate genome editing, disrupting the apoptotic resistance machinery of tumor cells. Another study presented a FUS-controllable CRISPR toolbox for cancer immunotherapy. They demonstrated that FUS-CRISPR-mediated telomere disruption primes tumors for CAR-T cell therapy and enhanced anti-tumor efficacy in vivo by reprogramming cancer cells and activating synNotch CAR-T cells.[56] This sonogenetic strategy, which enables inducible activation of multiple endogenous genes and base editing through ultrasound-mediated thermal induction, has been demonstrated potential for precise gene therapy and cell engineering applications by a hyper-efficient dCas12a system.[40] Furthermore, FUS-

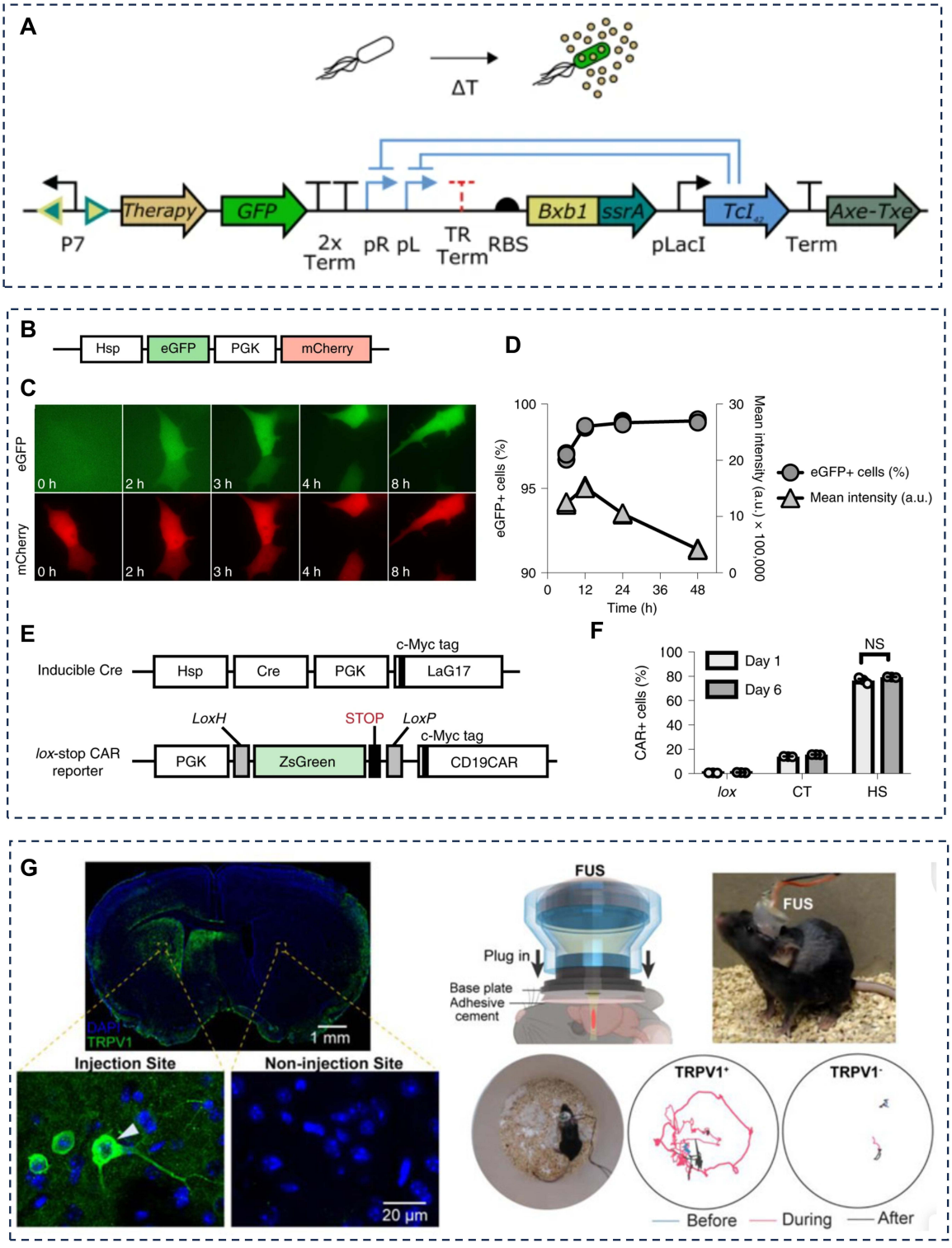

Figure 3 (**A**) Temperature-responsive state switches releasing αCTLA-4 or αPD-L1 nanobodies. Reproduced from Abedi MH, Yao MS, Mittelstein DR, et al. Ultrasound-controllable engineered bacteria for cancer immunotherapy. *Nat Commun*. 2022;13(1):1585.[44] (**B**) Schematics of the dual-promoter eGFP reporter. (**C** and **D**) The images of heat-induced eGFP expression and the percentages of eGFP expression cells and mean fluorescence intensities in T cells containing the dual-promoter reporter heated at 43°C for 15 min. (**E**) Schematics of transgenes: inducible Cre and *lox*-stop CAR reporter. (**F**) Inducible CAR expression in Jurkat cells hosting the *lox*-stop CAR reporter alone (*lox*), or both transgenes under heat stimulation (HT) or without heat stimulation (CT). Reprinted from Journal of Nature Biomedical Engineering, 5, 11, Wu Y, Liu Y, Huang Z, et al. Control of the activity of CAR-T cells within tumours via focused ultrasound. 1336–1347,Nat Biomed Eng. Copyright 2021 with permission from Elsevier.[41] (**G**) Expression of TRPV1 in the mouse brain after the viral injection, experimental setup for in vivo sonothermogenetic stimulation of the striatum in freely moving mice, and representative trajectories recorded in TRPV1 and TRPV1$^{+/-}$mice before (blue), during (red), and after (gray) FUS stimulation. Reprinted from Journal of Brain Stimulation, 4, 14, Yang Y, Pacia CP, Ye D, et al. Sonothermogenetics for noninvasive and cell-type specific deep brain neuromodulation. Brain Stimul. 790-800,Nat Biomed Eng. Copyright 2021 with permission from Elsevier.[55]

Abbreviation: NS, No significance.

mediated hyperthermia effectively altered the tumor physical microenvironment by increasing blood flow velocity and oxygen saturation in liver tumors, reshaping the extracellular immunosuppressive environment. The combination of these effects demonstrates synergistic therapeutic outcomes across various tumor models.[57]

Therapy utilizing engineered bacteria controlled by the FUS thermal effect is currently under investigation. TcI42 serves as a thermal transducer due to its significant induction at 42 °C while maintaining low baseline activity.[42–44] In a study conducted by Chen et al, the expression of the IFN-γ gene was examined following FUS thermal therapy. The IFN-γ gene was inserted into the multiple cloning sites under the PL and PR tandem promoters and then transformed into *E. coli MG1655*. Upon colonization of *E. coli* into the tumor, stable moderate temperatures from periodic FUS irradiation led to the relief of TcI repression, consequently triggering the expression of IFN-γ. This process promoted apoptosis of tumor cells and activated $CD4^+$ and $CD8^+$ T cells, thus enhancing anti-tumor immune therapy.[42] To achieve stable and long-term effect, Abedi et al introduced Bxb1 (A serine integrase that catalyzes inversion at a specific promoter, thus changing the transcription direction) under the control of thermally inducible promoters regulated by the TcI42 repressor to modify the gene circuit output, enabling the expression of αCTLA-4 or αPD-L1. The circuit containing αCTLA-4 or αPD-L1 in the engineered bacteria remains inactive at physiological temperatures. When the temperature caused by the FUS reaches 42°C or 43°C, the release of TcI42 repression triggers Bxb1 expression, which then catalyzes the inversion of attP and attB sites flanking the P7 promoter, allowing for the subsequent expression of αCTLA-4 or αPD-L1 for tumor therapy.[44] Additionally, in order to guide the ultrasound to irradiate intratumoral engineered bacteria, GVs are constructed on the ultrasound-thermal-responsive engineering therapy bacteria. Under high pressures, GVs collapse results in a loss of contrast signals, providing immediate imaging feedback to the operator during treatment.[58]

3.2 Sono-Thermal Transient Receptor Potential Channel Activation

In addition to the activation of promoters by ultrasound thermal effects, sono-thermogenetics based on FUS thermal therapies has also been developed. Transient receptor potential vanilloid 1 (TRPV1), a member of the thermosensitive transient receptor potential channel family, is highly expressed in peripheral neurons and brain regions, and is sensitive to temperature. When the temperature induced by the FUS reaches approximately 42 °C,[59] surpassing the thermal activation threshold of TRPV1, neurons containing TRPV1 become active, leading to significant calcium influx and changes in locomotor behavior without triggering inflammatory or apoptotic responses (Figure 3G). This indicates that TRPV1- and FUS-mediated sonogenetic neuromodulation can effectively and noninvasively control specific cell types in the deep brain with temporal precision.[55,60]

4. Sono-Mechanical Activation

The integration of ultrasound as an activation method with mechanosensitive ion channels represents a significant advancement in the field of ultrasound medicine. Researchers have discovered that activation of a bacterial mechanosensitive ion channel of large conductance (MscL) expressed in mammalian cells can be induced by localized membrane stress, which is influenced by the actin cytoskeleton.[61] Ibsen et al[62] found that Caenorhabditis elegans (C. elegans) show insensitivity to mechanical and temperature changes induced by low-pressure ultrasound, but respond behaviorally to a single pulse of low-pressure ultrasound in the presence of microbubbles. This study highlighted the significant role of mechanosensitive ion channels in ultrasound stimulation mechanisms, offering a non-invasive method for activating genetically targeted neurons. Several mechanosensitive ion channels, such as two-pore domain K^+ channels (K2Ps), Piezo1, MEC-4, transient receptor potential A1 (TRPA1), MscL, and voltage-gated Na^+ and $Ca2^+$ channels, have been identified as playing a role in cellular responses to ultrasound.[54,63–67] Among these channels, Piezo1 and MscL have been particularly well studied in the fields of ultrasound. Next, we discuss the design and applications in details, including cancer therapy and neural activity (Figure 4).

4.1 Cancer Therapy

Imbalance of ion channel proteins and transporters can lead to cell swelling and oncosis, suggesting a potential avenue for tumor therapy. Originally located on the cell membrane of bacteria, mechanosensitive channel proteins can be chemically or genetically modified to respond to various stimuli, such as ultrasound, thereby influencing cellular calcium balance and inducing cell death. Based on this mechanism, a logic AND-gated sonogene nanosystem utilizing cationic nanoliposomes was developed[68] (Figure 5A). Prolonged opening of MscL channels under continuous ultrasound exposure can lead to excessive calcium influx, triggering the calcium-related apoptosis pathway in cells. This process

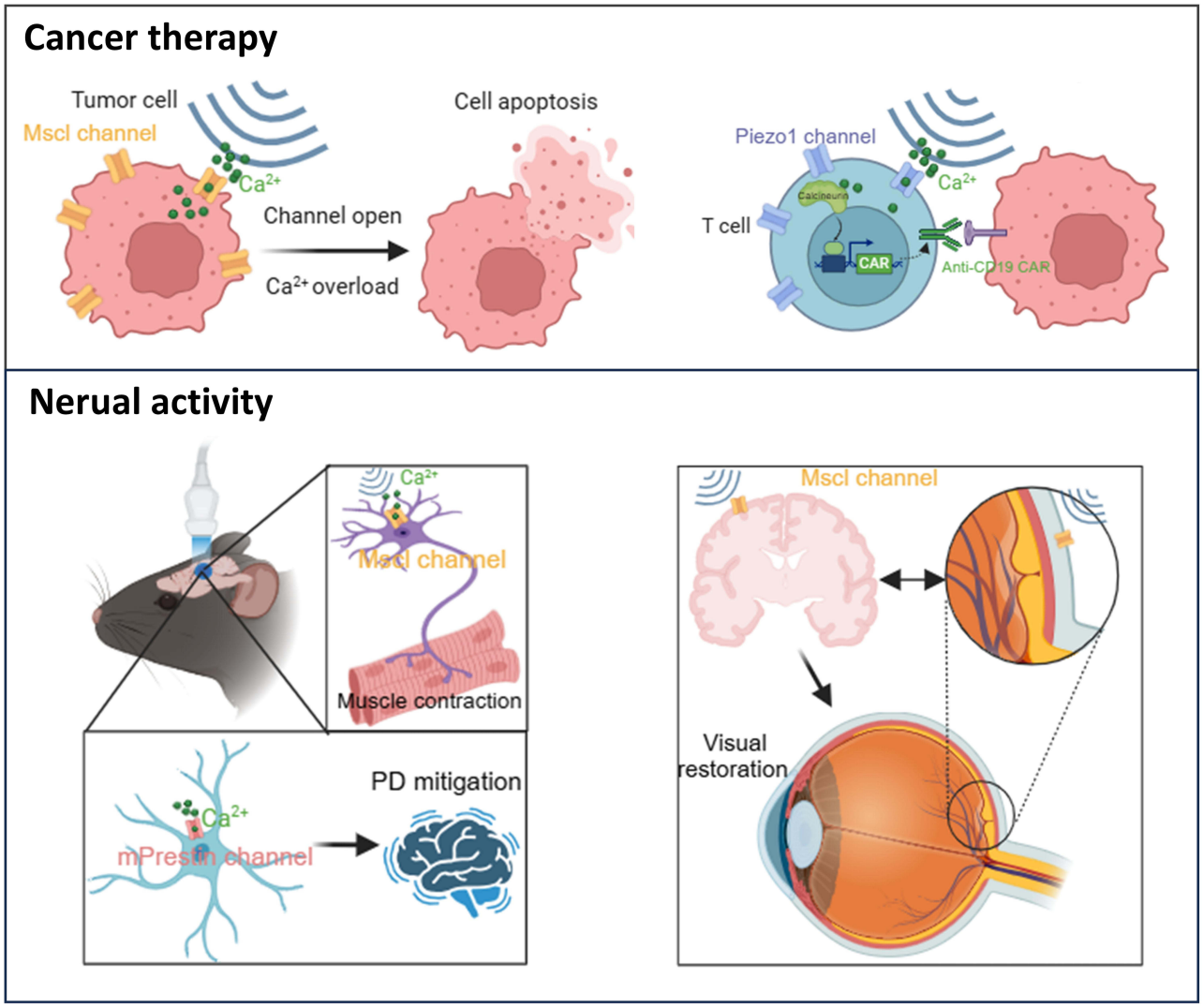

Figure 4 Application of sono-mechanical activation in medicine. Created in BioRender. Du, J. (2025) https://BioRender.com/k60r8g9.

induces mitochondrial dysfunction, ultimately inhibiting tumor growth and improving survival rates. Additionally, fragments generated from cell apoptosis serve as tumor-associated antigens, facilitating dendritic cell maturation and activation of $CD8^+$ T cells. This approach has been successfully applied in a melanoma model involving C57BL-6 mice, resulting in a significant anti-tumor immune response.[69]

Piezo1, as a mechanosensitive channel, was found to be highly expressed in pancreatic ductal adenocarcinoma (PDAC) cells and tissues and is closely related to poor disease-free survival and advanced clinical stages. The researchers used microbubbles to amplify ultrasound signals, leading to Piezo1 activation, calcium influx, mitochondrial dysfunction, and apoptosis in pancreatic cancer cells both in vitro and in vivo. The therapeutic effect of ultrasound with microbubbles was reduced when Piezo1 was knocked down, highlighting its importance in mediating the anticancer effects.[73]

Another research explored the use of ultrasound in combination with engineered genetic circuits to activate the Piezo1 ion channel as a mechanical sensor. The ultrasound-induced mechanical stimulation triggers the activation of Piezo1, leading to calcium influx which in turn activates the calcium-sensitive phosphatase calcineurin. Calcineurin then dephosphorylates a transcription factor of activated T-cells, allowing it to translocate to the nucleus. Once in the nucleus, this transcription factor activates a response element that drives the expression of specific target genes. This innovative system was successfully demonstrated in controlling Jurkat T-cell lines and primary human T cells for potential applications in cancer immunotherapy.[74]

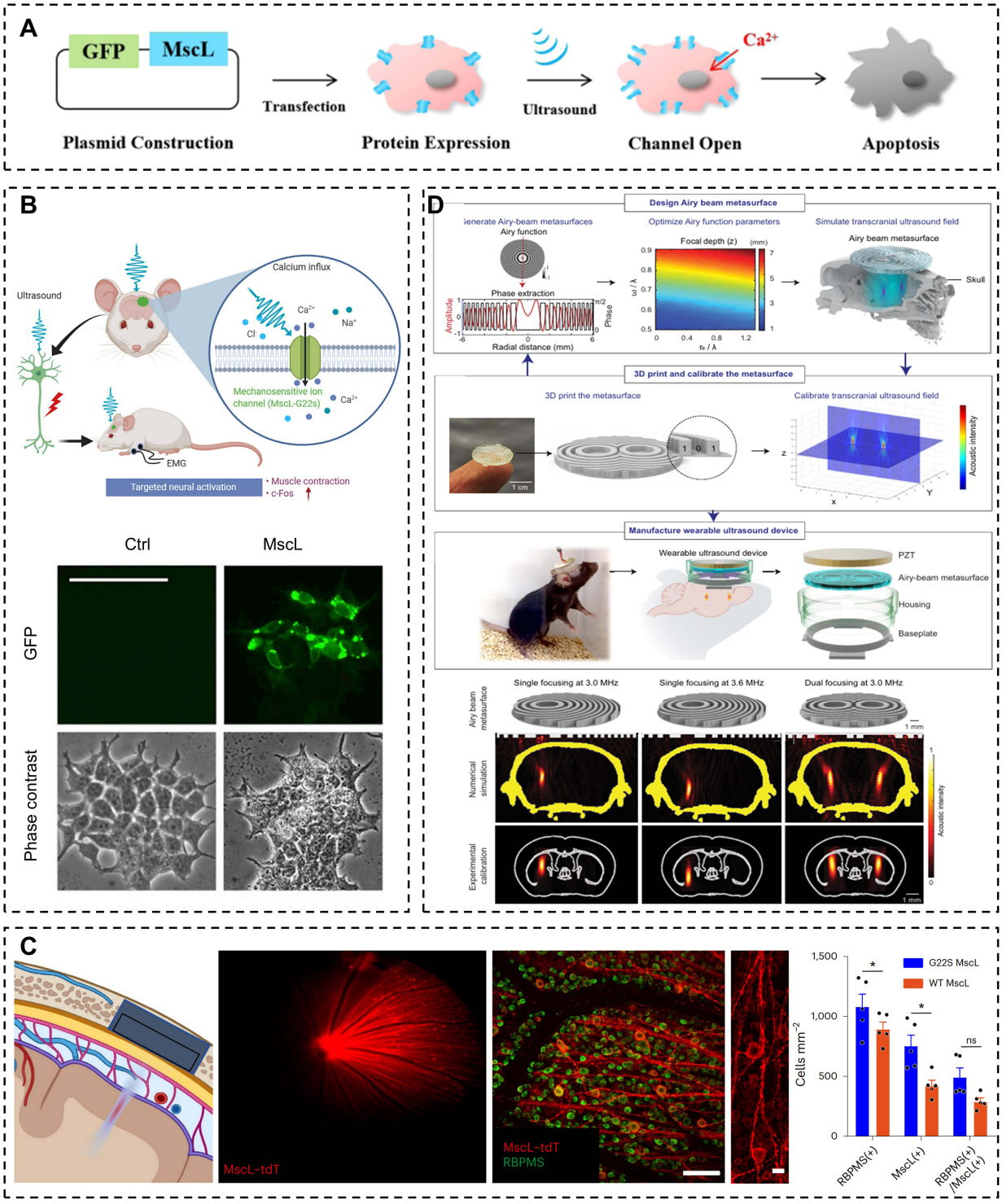

Figure 5 (**A**) Brief schematic diagram of the logic AND-gated sonogenetics strategy. Reprinted with permission from Wang T, Wang H, Pang G, et al. A logic AND-gated sonogene nanosystem for precisely regulating the apoptosis of tumor cells. ACS Appl Mater Interfaces. 2020;12(51):56692–56700. Copyright 2020, American Chemical Society.[68] (**B**) Non-invasive ultrasound triggered neural activation in MscL expressing regions. Used with permission of Cell Press from Qiu Z, Kala S, Guo J, et al. Targeted neurostimulation in mouse brains with non-invasive ultrasound. Cell Rep. 2021;34(1):108595. Permission conveyed through Copyright Clearance Center.[70] (**C**) Concept of visual restoration using US matrix arrays implanted in a cranial window for localized US neuromodulation of the primary visual cortex in humans, retinal fundus images of MscL–tdTomato expression, and the Density of RBPMS-positive, MscL-positive and double labelled cells. Reprinted with permissions from Cadoni S, Demené C, Alcala I, et al. Ectopic expression of a mechanosensitive channel confers spatiotemporal resolution to ultrasound stimulations of neurons for visual restoration. *Nat Nanotechnol*. 2023;18(6):667–676.[71] (**D**) The design of Wearable ultrasound device using Airy-beam holographic metasurfaces. Reproduced with permission from Airy-beam holographic sonogenetics for advancing neuromodulation precision and flexibility. Proc Natl Acad Sci U S A. 2024;121(26):e2402200121. https://creativecommons.org/licenses/by-nc/4.0/. [72] * $P<0.05$; **** $P<0.001$.
Abbreviation: ns, no significance.

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4.2 Neural Activity

Stimulating neuronal activity and signaling through physical intervention is a potent method for enhancing brain functions. Ultrasound-based stimulation shows promise as it has the potential to access deep brain structures in a non-invasive manner.[75,76] Some researchers have demonstrated the expression of MscL-G22S, a variant with a lower gating threshold compared to the wild-type, exclusively in specific cell types within the brain. It has been observed that low-intensity ultrasound can significantly activate these MscL-G22S-expressing neurons by inducing calcium influx, as well as evoke electromyogram responses in vivo. When neurons in the right dorsomedial striatum (DMS) of mice were engineered to express MscL-G22S and subsequently stimulated by ultrasound, researchers observed significantly greater neuronal activation than those not expressing the channel. This increased activation was specific to the right DMS and did not extend to the other regions[70] (Figure 5B), which makes it possible for non-invasive and repetitive treatment targeting deep brain regions. Furthermore, ultrasound-based MscL have also been investigated for visual restoration. Studies have shown that activating the MscL-G22S in retinal or cortical neurons led to rapid responses with millisecond latencies. These findings hold promise for advancing high-resolution visual restoration at the cortical level[71] (Figure 5C). Additionally, Piezo1 was demonstrated as a major mediator of ultrasound-induced neuronal activity and behavioral responses. Using a conditional Piezo1 knockout (P1KO) mouse model, researchers found that P1KO mice exhibited diminished limb movement, muscle electromyogram responses, and calcium signaling in the motor cortex upon ultrasound stimulation.[77] Moreover, a recent study found a mechanosensitive ion channel TRPA1, which act as an important role in synaptic modulation. The authors showed that low-frequency ultrasound activated TRPA1 channels in astrocytes, causing calcium influx and subsequent glutamate release through Best1 channels. This released glutamate activates N-methyl-D-aspartic acid receptor (NMDA) receptors in neighboring neurons, leading to motor behaviors and neuronal responses in vivo and in vitro.[64] These findings highlight crucial roles of mechanosensitive ion channels in mediating ultrasound neuromodulation and suggest their potential for targeted brain stimulation in neurological and psychiatric treatments.

Parkinson's Disease (PD) is a prevalent neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra. Researchers have long focused on investigating methods to improve the ultrasound-responsive properties of specific neurons to effectively activate dopaminergic neurons in the substantia nigra for the treatment of PD. They utilized an engineered US-sensing protein, mPrestin, to express in the dopaminergic neurons of the substantia nigra in mice model with PD. Their findings showed that repetitive neuronal stimulation through ultrasound could enhance the expression of neurotrophins, leading to improved functional behavioral recovery in the PD mice and alleviating their symptoms. The method utilized in this study involved low mechanical index (MI) and I_{spta} levels, which were significantly below the upper limit set by the Food and Drug Administration (FDA) for clinical ultrasound imaging. The procedure did not involve microbubbles and used low acoustic pressure (0.5 MPa), ensuring its safety. Furthermore, the absence of microglia, macrophages, apoptotic cells, and erythrocytes in the treatment area confirmed the biocompatibility of the technique within the tissue in vivo.[78]

The advancement of wearable ultrasound devices, in conjunction with synthetic biology, has enhanced the adaptability of ultrasound neural control. Airy-beam holographic sonogenetics (AhSonogenetics) utilizes Airy beams and 3D-printed metasurface-based wearable transducer technology to achieve precise and flexible sonogenetic neuromodulation in specific striatum subregions (Figure 5D). This technology allows for the unilateral and bilateral activation of neurons, offering potential targeted interventions for a range of neurological diseases, such as Parkinson's disease.[72]

4.3 Acoustic Applications of Gas Vesicles (GVs) in Ultrasound Medicine

The utilization of gas vesicles (GVs) has significantly promoted ultrasound applications for both imaging and therapy. Originally discovered as components in cyanobacteria, GVs consist of amphiphilic protein shells, with typical cylindrical dimensions ranging from 45 to 250 nm in width and 100–600 nm in length.[79] These structures are permeable to gas while effectively repelling liquid water from the surrounding media, enabling gas to diffuse freely through a 2 nm protein shell. As a result, no pressure gradient exists between the interior and exterior of GVs, which allows them to maintain inherent stability despite their nanometer scale.[80] A study conducted in 2014 revealed that biosynthetic GVs derived from Anabaena flos-aquae (Ana) and Halobacterium salinarum NRC-1 (Halo) can serve as effective ultrasound contrast agents both intracellularly and in vivo, illustrating their potential as acoustic biomolecular contrast agents for ultrasound applications.[80] Subsequently,

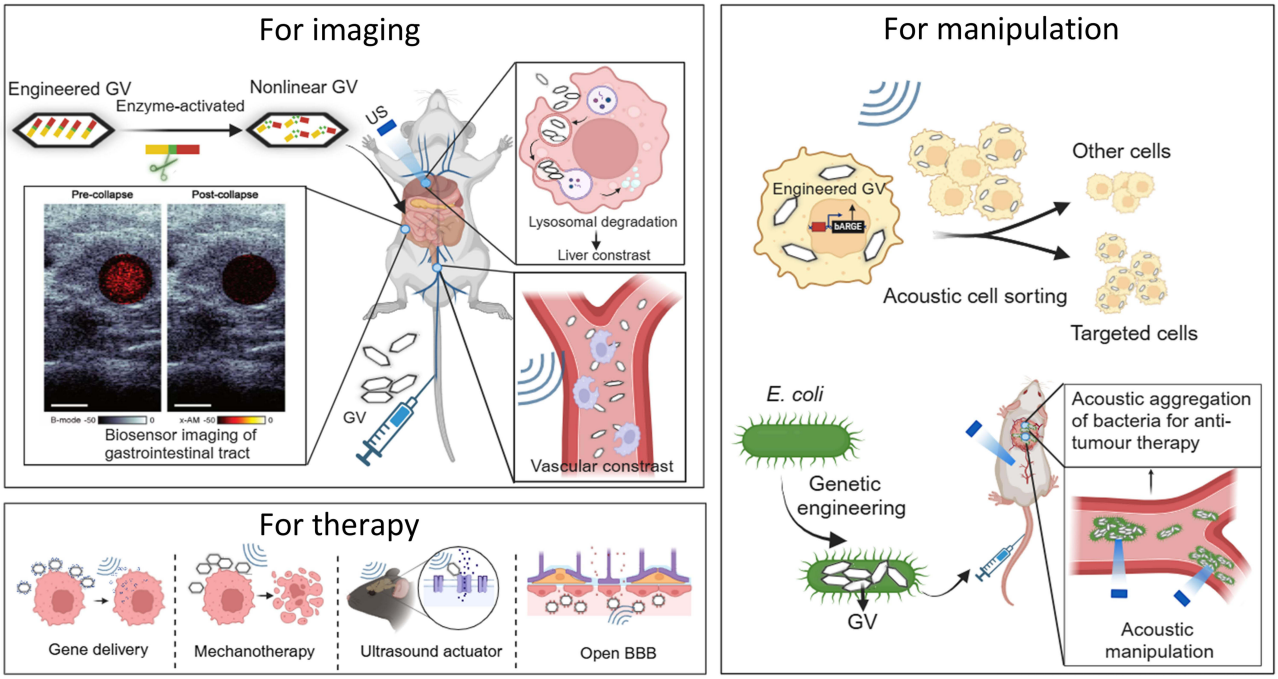

Figure 6 Gas vesicles for imaging, acoustic manipulation and therapy in ultrasound medicine.[84] Copyright 2020, the Author(s). Created in BioRender. Du, J. (2025) https://BioRender.com/j41sk3v.

a series of genetically encoded GVs-based investigations have emerged, and their combined application with ultrasound has expanded the scope of ultrasound imaging, therapy, and acoustic genetic manipulation[81–83] (Figure 6).

5. Acoustic Imaging of GVs

Visualizing molecular and cellular processes occurring deep within living organisms are fundamental to our study of basic biology and disease. In comparison to microbubbles confined within the vasculature, small-sized GVs have the ability to enter the local tumor and into the cell, then generate a strong ultrasound contrast signal under ultrasound irradiation, which provides the basis for ultrasound-based imaging, especially functional imaging. Certain studies utilize biosynthetic GVs for visualizing and quantifying phagocytic clearance and lysosomal degradation in vivo.[85] Intravenously injected GVs are quickly absorbed by the liver, where they are taken up by macrophages and subsequently broken down into lysosomes through phagocytosis. This process is characterized by a two-compartment pharmacokinetic model involving the blood and liver. Researchers utilized this model to track disease progression in two liver disease models: clodronate-induced macrophage deficiency and diet-induced nonalcoholic fatty liver disease (NAFLD). They observed that in diseased mice, the rates of uptake and degradation were lower than those in healthy mice, attributed to a decrease in macrophage population. Furthermore, the phagocytic and lysosomal functions were suppressed in NAFLD mice.[85] In another research, biosynthetic GVs were used to track natural killer (NK) cells. Following the enhancement of NK-cell accumulation in tumor with a widely acknowledged booster, the ultrasound signal significantly increased at both 3 hours and 24 hours after infusion, indicating the effective tracking of the adoptive NK cells using this approach.[86]

The outer shell of biosynthetic GVs is primarily composed of GV proteins A (GvPA) and C (GvPC). GvPA forms the main material of the primary GVs shell, while GvPC serves as the outer scaffold that impacts the shape and structural integrity. As a genetically encoded material, GvPC-based designs through genetic engineering allow for the modification of mechanical, acoustic, surface, and targeting properties of GVs to achieve various applications with ultrasound control.[83] Some researchers developed genetically engineered GVs-based biosensors to detect protease activity for noninvasive imaging, allowing for dynamic changes in ultrasound contrast with specific proteases[84] (Figure 7A-C). Through engineering variants of the gas vesicle protein C, they created GVS_{ClpXP}, genetically engineered GVs with

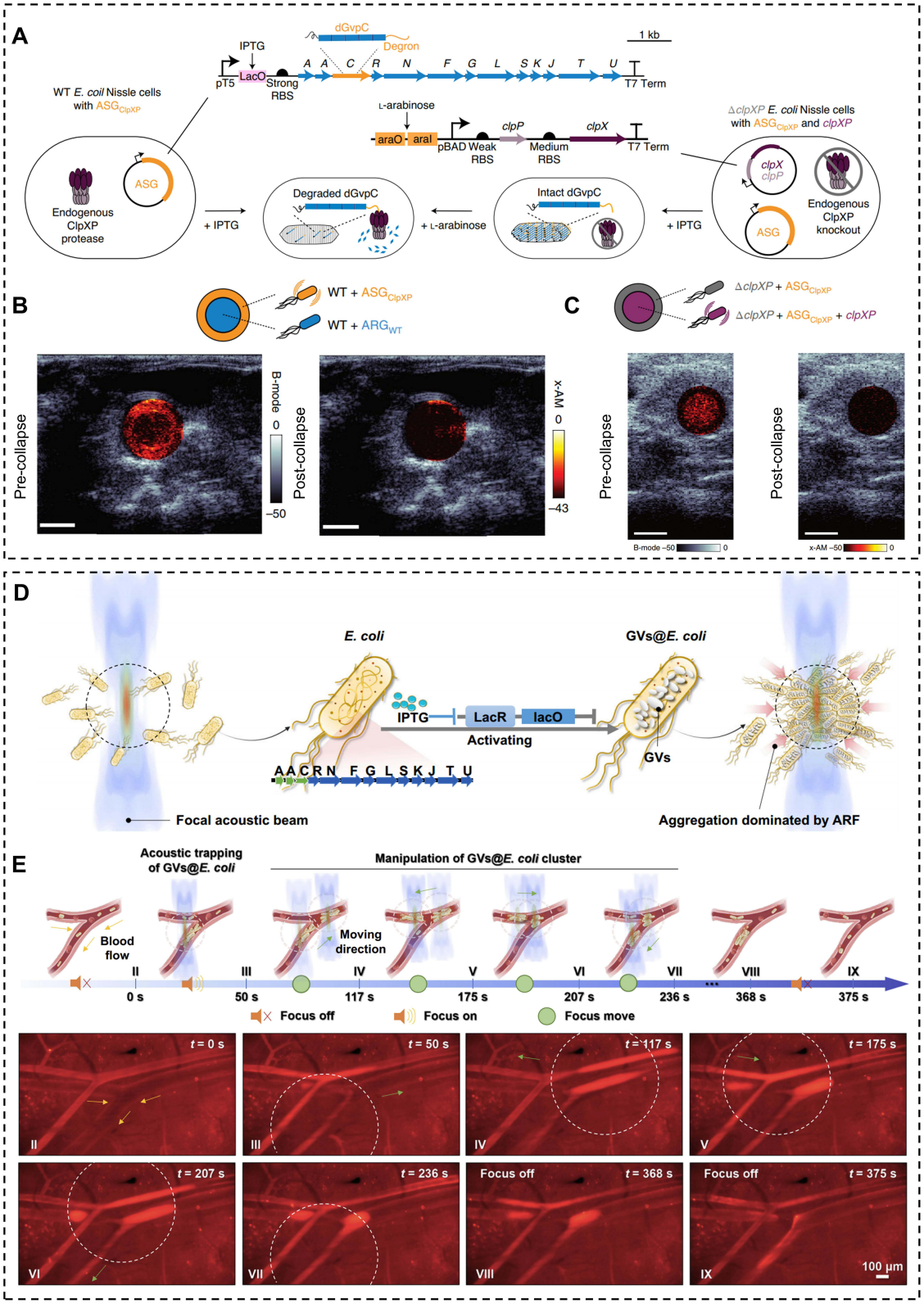

Figure 7 (**A**) Schematic of *E. coli Nissle* cells expressing the ASG construct for ClpXP. (**B**) Transverse ultrasound image of a mouse whose colon contains WT Nissle cells expressing ARGWT at the center of the lumen and the same strain expressing ASGClpXP at the periphery of the lumen. (**C**) Transverse ultrasound image of a mouse whose colon contains ΔclpXP Nissle cells expressing ASGClpXP with l-arabinose induction of ClpXP protease expression at the center and without l-arabinose induction at the periphery of the lumen. Used with permission of Nature Research from Lakshmanan A, Jin Z, Nety SP, et al. Acoustic biosensors for ultrasound imaging of enzyme activity. Nat Chem Biol. 2020;16(9):988–996. Permission conveyed through Copyright Clearance Center.[84] Copyright 2020, the Author(s). (**D**) Schematic of the acoustic trapping of GVs@*E. coli*. (**E**) Manipulating the GVs@*E. coli* cluster selectively through the fork of the vessels. Reprinted with permission from Yang Y, Yang Y, Liu D, et al. In-vivo programmable acoustic manipulation of genetically engineered bacteria. Nat Commun. 2023;14(1):3297.[50]

nonlinear ultrasound contrast that can be activated by protease activity, and successfully enabled in vivo imaging of the gastrointestinal tract in mice.

5.1 Acoustic Therapy of GVs

GVs can act as seeds for bubble formation and cavitation under ultrasound irradiation,[87,88] which are utilized as a non-viral delivery system for gene-editing purposes.[89–91] In a study, a plasmid containing sgRNA targeting exon 3 of the Cdh2 gene was delivered to cancer cells using biosynthetic GVs in combination with ultrasound-mediated cavitation. The results suggest that the use of GVs along with ultrasound irradiation could enhance gene transfection efficiency and potentially slow down tumor growth.[90]

Another strategy is to use biosynthetic GVs as an essential acoustic-mechanical transducers, a masked 2-furylcarbinol mechanophore was employed for mechanically induced molecular release. In the presence of biosynthetic GVs, FUS activates the mechanophore under biocompatible conditions, leading to the release of covalently bound fluorogenic and therapeutic cargo molecules.[92] In addition, biosynthetic GVs are not only utilized for opening the blood–brain barrier (BBB),[93] but also function as localized acoustic actuators and amplifiers to reduce the threshold of ultrasound intensity required for precise neurostimulation. When primary neurons were engineered to express the mechanosensitive ion channel MscL-EYFP or EYFP alone, it was observed that MscL-EYFP+GVs exhibited a calcium response to ultrasound that was significantly stronger compared to EYFP+GVs. This highlights the potential of using biosynthetic GVs as ultrasound actuators to lower the threshold for ultrasound-induced neuronal activation, resulting in localized stimulation effects.[94] Furthermore, the combination of biosynthetic GVs with bacteria can be utilized to target solid tumors and activate GVs through ultrasound-induced cavitation, leading to mechanical destruction of tumor tissue in vivo. This approach has shown promise in enhancing cancer immunotherapy, as the combined treatment with checkpoint inhibitors and FUS has resulted in a significant increase in survival time in mice.[81]

Some researchers also study smaller genetically encoded GVs, approximately 50 nm in size.[95] These small GVs have the ability to penetrate lymphatic endothelial cells and potentially even the blood–brain barrier,[96] offering promising implications for the treatment of neurological diseases.

5.2 Acoustic Manipulation of GVs

In addition to imaging and treatment, the combined biosynthetic GV technology offers a directional approach for acoustic manipulating cells using acoustic signals in live organisms. Previous studies have explored the manipulation of non-living objects with ultrasound, such as glass spheres within a pig bladder.[97] The weak ARF on small-sized cells,[98] which is proportional to the third power of the cell radius, along with the similar acoustic impedance between cells and the surrounding medium,[99] makes ARF manipulation of living cells challenging. However, cells that are genetically engineered to express GVs could potentially experience a significantly altered radiation force as a result of changes in their acoustic properties. This could enable selective acoustic manipulation and potentially open up new possibilities for genetic manipulation. Some researchers focus on studying the precise and targeted manipulation of bacterial cells using acoustic trapping with ARF.[47,100] By generating a powerful ARF through a transducer, they are able to trap genetically engineered GV bacteria and guide them along a predetermined path using programmable pulses. This method allows for the manipulation of GV clusters within complex blood vessels, enabling them to be controlled to oscillate, remain stationary at specific locations, and selectively navigate through vessel bifurcations[50] (Figure 7D and E). Furthermore, the expression of GVs enables the selective acoustic manipulation and sorting of mammalian cells according to their genotype.[100] In a study where GVs were expressed in Salmonella typhimurium YB1, with tumor lysing properties, the experimental group demonstrated the most effective anti-tumor therapy by utilizing ultrasound to trap these engineered bacteria and enhance their accumulation in tumors.[50]

6. Conclusions and Future Outlook

Ultrasound, as a deep penetration and precise activation method, has been extensively studied for imaging and therapy. The intersection of ultrasound and synthetic biology aims to achieve precise non-invasive control over specific cells by engineering them to effectively respond to both the thermal and mechanical effects of ultrasound. This approach

significantly contributes to the field of ultrasound-based medicine. In this review, we discuss the activation mechanism of ultrasound, and outline key control strategies in sonogenetics and gas vesicle, including sono-thermal promoter switch, sono-thermal sensitive receptor potential channel activation and sono-mechanical activation. We also explore the applications of these strategies in various medical fields such as tumor therapy, immune therapy, neural activity modulation, visual restoration, and acoustic manipulation.

Ultrasound-based activation strategies in synthetic biology are an emerging approach that enables non-invasive modulation and treatment. However, significant efforts are still necessary before these promising technologies can have a substantial impact on medicine. The combination of ultrasound with synthetic biology has shown potential in neurostimulation and the treatment of neurological disorders. While there have been limited studies concerning the application of sonogenetic tools for modeling neuropathologies, further exploration in this area could offer new opportunities for developing animal models of human neurological diseases that are unattainable through other methods. This includes the ability to target specific signaling and metabolic pathways, manipulate defined cell types, and regulate the progression, severity, and duration of pathological states. Accurately mimicking these pathological conditions is crucial for investigating the underlying causes of neurological diseases, studying disease progression, and evaluating treatment effectiveness. Additionally, there are still unexplored disorders related to brain function, particularly neurodegenerative diseases and psychiatric disorders like memory impairment, depression, and anxiety. Developing treatments for these conditions is essential for promoting mental health in modern society. Additionally, standardizing transducer designs to establish defined ultrasound parameters is critical for researchers, as it could facilitate comparisons of study effectiveness in physiological contexts. Current research in sono-synthetic biology is primarily focused on developing sonogenetic switches in the laboratory phase. However, further validation of safety and efficacy, as well as more in-depth mechanistic studies, are necessary to enhance the therapeutic potential of these technologies. Transitioning these advancements to clinical biomedical research will require time and continued efforts.

In conclusion, ultrasound-based activation strategies in sonogenetics and gas vesicle has shown promising results in both ultrasound imaging and therapy, expanding the application of ultrasound in medicine. Further research is necessary to fully comprehend the diagnostic and therapeutic capabilities of this innovative technology. It is anticipated that ultrasound-based activation strategies will play a significant role in advancing the biomedical field in the future.

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Disclosure

The authors declare that there is no conflicts of interest in this work.

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