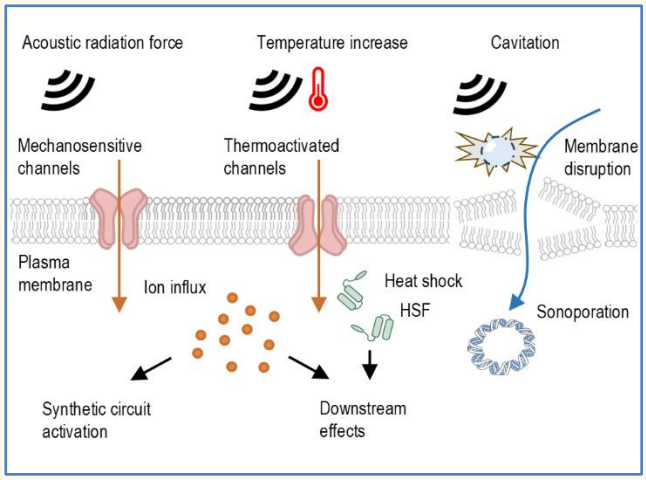
Ultrasound-responsive mammalian cell synthetic biology

Filip Ivanovski, Vid Jazbec, Nina Varda, Roman Jerala, Mojca Benčina

***Abstract*— Sonogenetics is developing into a powerful tool in synthetic biology. The coupling of ultrasound with genetically engineered effectors enables non-invasive and precise control of cellular and molecular processes. Building on established techniques such as optogenetics, it overcomes the limits of tissue penetration and invasiveness, making it a promising tool for both research and therapeutic applications. Recent advances in acoustic contrast agents, such as microbubbles and gas vesicles, have improved the mechanical effects of ultrasound on cells, extending its application to various biological systems. This review highlights recent advances and challenges, such as standardization of parameters and understanding of underlying mechanisms, and outlines future directions for ultrasound-guided cellular control.**

***Index Terms*— ultrasound, synthetic biology, sonogenetics, mechanosensitive ion channels**



I. Introduction

Advances in technologies that enable precise and non-invasive manipulation of cellular and molecular processes have transformed biomedical research. Technologies such as optogenetics, magnetogenetics, and sonogenetics have opened new possibilities for manipulating biological systems. In contrast to chemically inducible systems, which are commonly used for synthetic biology applications and are limited by their pharmacokinetics and off-target effects, these technologies allow remote control of cellular processes with spatial and temporal precision through external stimuli. This enables improved diagnostics, therapy and basic research opportunities. Among these modalities, ultrasound (US) has proven to be a highly versatile technique for imaging and modulation that allows non-invasive access to deeper tissues and precise control of biological processes.

Optogenetics first established itself as an effective tool due to its ability to excite specific neurons or other cells via light-sensitive ion channels (rhodopsin's [1]) and other light-sensitive proteins (e.g. LOV2, CRY2 [2]), allowing researchers to analyze neuronal circuits with an unmatched degree of specificity [3], [4]. However, the low penetration depth of light into tissue — due to scattering at a depth of 5 mm — requires invasive methods for deep tissue applications, making it less suitable for clinical use in patients [5], [6]. Magnetogenetics, on the other hand, uses systems conjugated with nanoparticles. This enables two distinct modes of operation, namely the heating of nanoparticles or mechanical force through magnetism. [7] Magnetogenetics benefits from the ability of magnetic fields to penetrate deep tissue non-invasively, but also suffers from limited spatial resolution and slower kinetics compared to optogenetics. In addition, most systems require the delivery and precise localization of magnetic nanoparticles,

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Highlights

- **We highlight the progress of sonogenetics as a non-invasive tool for the precise control of cells by utilizing ultrasound-responsive effectors.**
- **Ultrasound, enhanced by acoustic contrast agents, effectively modulates mechanosensitive ion channels, enabling synthetic biology-driven gene regulation.**
- **Sonogenetics offers transformative potential for biomedical applications, including neuromodulation, regulation of immune responses and gene therapy, and paves the way for innovative, non-invasive therapeutic strategies.**

which can lead to biocompatibility issues, potential immune reactions and complex delivery methods. In addition, the need for strong magnets or magnetic field gradients adds to the complexity of implementation and limits scalability in clinical or in vivo applications [7], [8]. These limitations have led to other methods being explored, including sonogenetics.

Sonogenetics, the control of cellular processes by US waves using genetically engineered US-responsive mediators, has attracted considerable attention due to its non-invasiveness, safety, wide application in clinical theranostics and deep tissue penetration. As a mechanical wave that penetrates biological tissue with high efficiency and without significant attenuation, US operates over a wide frequency range, enabling its application on a variety of temporal and spatial scales [9]. Compared to light, which can penetrate only millimeters into soft tissue or even less into bone tissue [6], [10], US can deliver energy several centimeters deep into tissue with a spatial resolution equal to its wavelength. For example, with US at 15 MHz, the brain can be expected to be imaged 2 cm deep with a resolution of 100 μm [11]. With the introduction of focused US (FUS) guided by magnetic resonance imaging (MRI), it is now possible to precisely target specific tissue volumes, significantly increasing therapeutic and research potential [12], [13].

The combination of ultrasound and genetic engineering—the hallmark of sonogenetics—offers remarkable precision in influencing cell activity. Genetic engineering enables the introduction of US-sensitive mediators into the desired cells, making them more sensitive to US stimulation. Such a combination allows researchers to achieve cell-type selectivity and limit off-target effects. The extension of sonogenetics to the field of neuroscience holds immense transformational potential as it provides a non-invasive solution for the study and treatment of neuronal functions and dysfunctions [14]. Neurodegenerative diseases, which are usually treated by alleviating symptoms, could greatly benefit from this type of approach.

In this review, we will discuss recent advances in the field of US modulation of mammalian cells and systems. Synthetic biology, which deals with the modulation of cell function, has so far shown the potential of US technology. Due to the various effects that US has on cells, e.g., acoustic radiation force, heating and cavitation, researchers have developed suitable molecular systems to either enhance the effect of US or use it to modulate other cellular activities.

II. HARNESSING ACOUSTIC RADIATION FORCES

The ability to respond to mechanical stimuli is one of the fundamental aspects of life. Mechanical stimuli are received at the cellular level by large structures such as the cell membrane and cytoskeleton and prompt cell response via proteins directly (such as integrins, cell adhesion molecules, etc.) or are converted into signals that can be interpreted by cells via mechanosensitive (MS) ion channels. There are many recognized families of mechanoreceptive ion channels. In bacteria, MscL and MscS are the best studied, while MscM is an example in archaea [15]. Eukaryotic cells possess multiple superfamilies of ion channels, of which some or all members can respond to mechanical forces (**Fig. 1A**). The Piezo channel [16] is a prime example, as its activation relies on the curvature of the surrounding membrane, which it detects through its three long blades made of transmembrane helices. Known MS channels also include members of the two-pore potassium channels (K2P), TREK [17] and TRAAK [18]. The transient receptor potential (TRP) channel superfamily includes several members that are mechanosensitive [19]. Although there are many potential MS channels, not all of them have been shown to be activated by ultrasound (**Table 1, Table S1**).

The use of sonogenetics in mammalian cells in the context of synthetic biology has primarily relied on channels that facilitate the influx of Ca^{2+} due to its strong binding affinity to protein structures. The effects of Ca^{2+} influx on cellular behavior can be divided into two types: ultrasound-stimulated native behavior and ultrasound-stimulated synthetic behavior. Most research has focused on neurons, as Ca^{2+} ions play a central role in their function.

The field of sonogenetics was established by a pioneering study in which ultrasound was used to alter the behavior of *C.elegans* via Ca^{2+} influx, which was initially attributed to TRP-4 channels [20]. The study also showed that microbubbles significantly enhanced the effect of ultrasound by amplifying acoustic responses. A subsequent study replicated these results but identified MEC-4 as the channel responsible for the observed effects [21]. In mice, expression of a mutant mechanosensitive channel, MscL-G22S [22], enabled non-invasive stimulation of specific neurons, as evidenced by upregulation of the c-Fos signaling pathway. Similarly, a mutant of prestin [23], a protein that is not an ion channel but can enhance mechanical effects on the membrane, was used to sensitize neurons to ultrasound stimulation [24]. In retinal cells, ultrasound stimulation activated neurons, although the specific channels involved were not defined [25]. In addition, higher frequencies of focused ultrasound were shown to activate transiently expressed Piezo channels in CHO and HEK293 cells, further highlighting the potential of sonogenetics to target different cellular systems [26], [27]. While most studies have focused on individual subpopulations of excitable cells such as

neurons, recent advances have highlighted how ultrasonic Ca^{2+} induced activation of various sections of brain neurons can lead to various behaviors in mice [28], [29].

TABLE I

Channel	Frequency (MHz)	Pressure/Intensity	Reference
Bacterial			
MscL	0.5, 2.25, 10, 15	0.047-1.6 MPa	[83], [84]
MscL G22S	0.5, 0.65, 0.9, 1, 2.5, 10, 15	0.025-1.6 MPa	[22], [83], [84], [85], [86], [87]
MscL I92L	2, 6, 30	0.25-3 MPa	[88], [89]
MscS	3.5	0.04-0.62 W/cm2	[90]
Worm			
TRP-4	2.25, 10	0.41-1 MPa	[20], [21], [91]
MEC-4	2.25, 10, 27.4	0.79-3 MPa	[21], [91], [92]
MEC-6	27.4	~3 MPa	[92]
Mouse			
TRPA1	0.35, 0.43	*In vitro*: 306.1 mW/cm2, *In vivo*: 702 mW/cm2	[93]
TRPC1	0.3, 0.67, 1.15, 1.125	4 MPa, 15 W/cm2	[13], [74]
TRPC6	1	Ispta: 0.19 W/cm2	[94]
TRPP1/2	0.3, 0.67	0-15 W/cm2	[74]
TRPM4	0.3, 0.67	0-15 W/cm2	[74]
Piezo1	0.111, 0.3, 0.5, 0.62, 0.67, 2, 2.25, 30, 43, 150	0.02-3 MPa, 15, 50, 90 W/cm2	[26], [27], [31], [74], [87], [95], [96], [97], [98]
Piezo2	0.62, 3.57	0.57 MPa, 11-743 W/cm2	[87], [99]
ASIC1a	1	*In vivo*: Ispta=5 mW/cm2, *in vitro:* Isppa=7.4 mW/cm2	[100]
VGSC	0.44, 0.67	< 1 MPa	[101]
NMDA	0.5	0.3, 0.6 MPa	[102]
Rat			
TRPV1	1.5, 1.7	0.7-1.3 MPa	[62], [63]
Piezo1	2	0.03-0.17 MPa	[98]
K2P	1, 43	Isata=50 mW/cm2, Isppa=50 W/cm2	[103], [104]
NR1A/2A/2B	0.5	0.3, 0.6	[102]
Bovine			
GPCR	1	0.0117 MPa	[105]
ASIC3	1	0.0117 MPa	[105]
Human			
TRPA1	1, 2, 6.91, 7	0.32-2.5 MPa	[106], [107]
TRPV4 F617L	1	0.06-1.8 MPa	[108]
TRPs and/or IP_3 receptor	38	0.394 MPa	[109]
TRPC1	0.3, 0.67	0-15 W/cm2	[74]
Piezo1	0.5, 1, 0.111	0.1764-0.66	[22], [26], [34], [110]
TREK-1	10	0.12, 0.24 MPa	[17]
TREK-2	10	0.12 MPa	[17]
TRAAK	2.25, 3.5, 5, 10	0.12 MPa, 0.01-4.3 W/cm2	[17], [111], [112]
$Na_v1.5$	10	0.425-1.75 MPa	[17]
BK_{Ca}	1	0.3 MPa	[113]
PANNEXIN-1	46	0.3-1.772 W/cm2	[114]

ISPTA spatial peak temporal average intensity
ISPPA spatial peak pulse average intensity
ISATA spatial average temporal average intensity

Apart from the cell-native effects of ultrasound, research in the field of synthetic biology has used it in conjunction with more complex circuits. The use of the Ca^{2+} increase achieved with ultrasound borrows heavily from concepts developed in optogenetics [30]. Elevated Ca^{2+} levels activate the NFAT (nuclear factor of activated T cells) transcription pathway, in which Ca^{2+}-bound calmodulin binds to calcineurin, a phosphatase that removes the phosphate groups from NFAT and allows it to translocate to the nucleus. The synthetic circuit that enables the transcription of the desired gene can be based either on the cell's own NFAT or on synthetic transcription factors derived from NFAT. The former utilizes NFAT response elements and was a proof of concept of such a sonogenetic approach (**Fig. 1B**) [31]. More advanced approaches use only the N-terminal domain, which is responsible for Ca^{2+}-dependent translocation into the nucleus, in combination with Cas [32] or TALE [33] DNA-binding elements (**Fig. 1C**). The latter has been used in combination with ultrasound and is effective both in vitro and as adoptive cells in mice [34]. As an implanted therapeutic cellular device, the ultrasound-activated cells were able to express sufficient IL-10 to alleviate DSS-induced colitis. Another design called CaDox, which relies on doxycycline alongside US-induced Ca^{2+}, has been shown to have shorter stimulation times for the production of gene interest [35]. The design optimizes the calcineurin binding to the NFAT domain of the transcription factor.

The optimization of acoustic force effects has extended beyond ultrasound parameters to acoustic contrast agents (**Fig. 1D**). The simplest of these are lipid microbubbles, already used in the study on *C. elegans*, where they were added to the medium without attaching to the cells [20]. Binding microbubbles to cells, as expected, enhances ultrasound effects [36]. In several studies, microbubbles bound to specific channels were used to increase their mechanosensitivity (**Table S1**). Other hollow structures that serve as contrast agents are gas vesicles (GVs). These hollow protein structures, which give microorganisms buoyancy, have gained interest in recent years as contrast agents for imaging [37], [38]. Recent studies have made progress in the binding of GVs to mammalian cells or even their expression in mammalian cells [39], [40], [41]. In addition to imaging, GVs have also been used to move cells in an acoustic field [42] or amplify the acoustic force exerted on cells [43]. Since the interaction with membranes was facilitated via integrins, they were able to indirectly open TRPV4 channels [44].

III. TEMPERATURE-RESPONSIVE SONOGENETICS

In addition to its primary role as a diagnostic imaging tool, ultrasound has also been used as a multifunctional therapeutic tool, particularly for thermal ablation [45]. With focused ultrasound (FUS), high-intensity acoustic energy can be delivered to specific tissue areas, where the energy absorption leads to a controlled temperature increase. This results in thermal coagulation and ablation of the pathological tissue with minimal invasiveness and without damaging healthy tissue [46].

When cells are exposed to heat stress, heat shock factors (HSF) are phosphorylated and form trimers. These trimers are

transported into the nucleus, where they bind to heat shock promoters and activate transcription [47] (**Fig. 2A**). Since FUS can locally increase tissue temperature, it was coupled with a heat-sensitive promoter to regulate gene expression. In several studies, a heat-sensitive promoter pHsp70b was used to spatially and temporally regulate the expression of the target gene [48], [49], [50], [51], [52]. While higher temperatures and longer stimulation durations result in higher expression levels of the target gene, a short exposure of 2 minutes at 43 °C is sufficient to achieve robust induction without damaging the tissue [52]. Heat-mediated control of gene expression has been used to improve the precision of chimeric antigen receptor (CAR) T cells. CARs under the control of a heat shock promoter in T cells were used to eradicate target tumor cells in vitro (FUS-CAR). However, to achieve sufficient expression, a one-hour incubation at 42 °C was required, resulting in a 40% decrease in cell viability [53]. When using a heat-inducible Cre recombinase to induce CAR expression, three five-minute stimulations at five-minute intervals with FUS that heated the tumor region to 43 °C were sufficient to induce CAR expression in mice, resulting in successful tumor eradication [54] (**Fig. 2B**). This approach enables the spatial activation of CAR T cells in restricted tissue regions, reducing the risk of on-target-off-tumor toxicity. By improving the promoter region and introducing a positive feedback loop that is activated when the CAR is engaged, an EchoBack CAR was developed. It has an even steeper activation

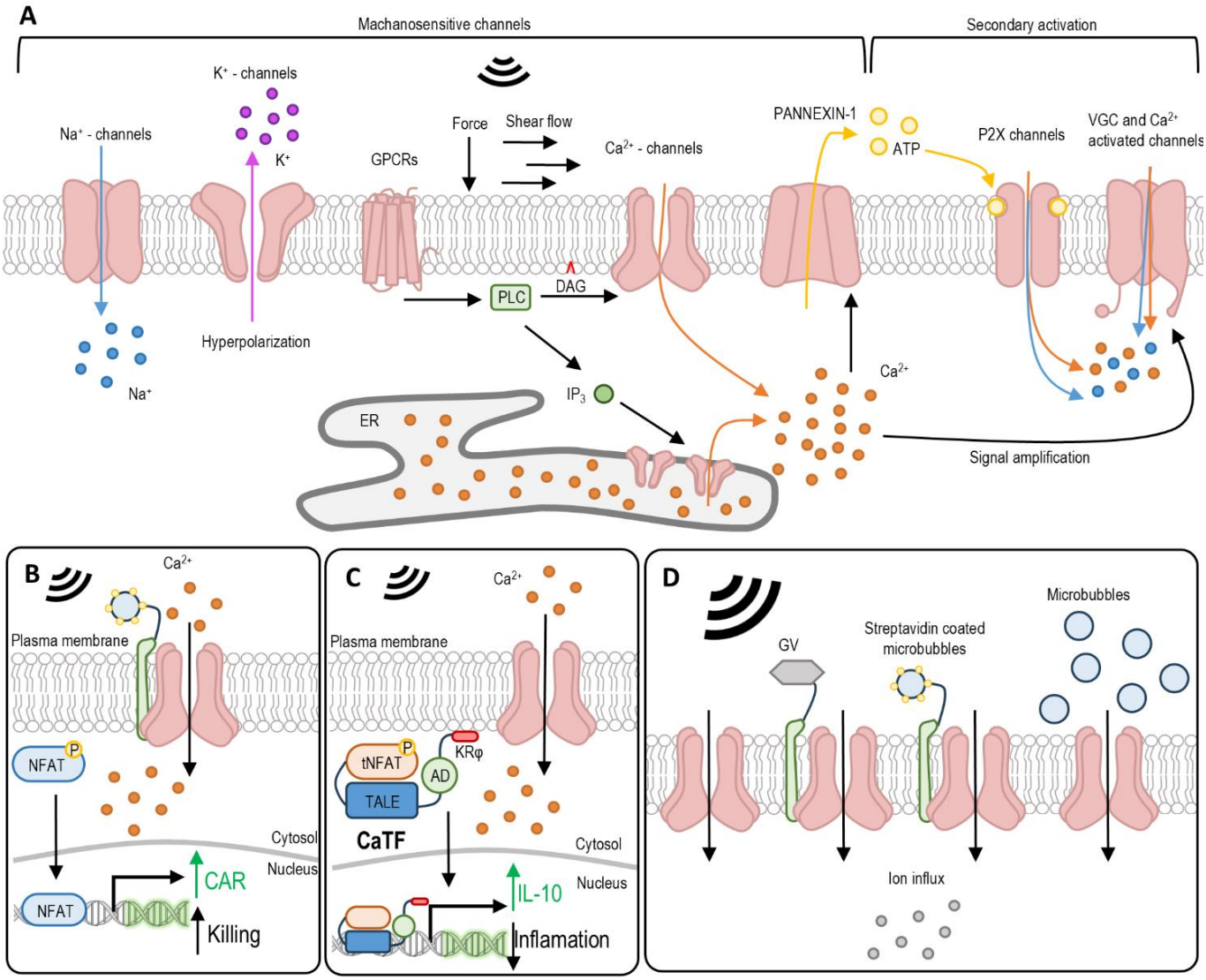

Fig. 1. **Mechanisms of sonogenetic modulation.** (**A**) Various mechanosensitive ion channels, including Piezo, TREK, TRAAK and TRPV, respond to ultrasound-induced membrane shape, leading to calcium influx and intracellular signaling. (**B**) Streptavidin-coated microbubbles bind to integrins or biotinylated membrane proteins and amplify ultrasound signals to mechanically activate Piezo1 ion channels. This leads to Ca^{2+} influx, which triggers the NFAT signaling pathway and promotes the expression of anti-CD19 CAR for cancer immunotherapy. [31] (**C**) Ultrasound stimulation induces a Ca^{2+} influx that causes the release of synthetic Ca^{2+}-responsive transcription factors (CaTF) from the plasma membrane and their translocation to the nucleus. This activates the expression of interleukin-10 (IL-10), which enables anti-inflammatory therapy. CaTF consists of truncated NFAT (tNFAT), a synthetic TALE DNA-binding domain, an activation domain (AD) and a Ca^{2+}-responsive membrane-anchoring peptide (KRφ).[34] (**D**) Gas vesicles and microbubble-based contrast agents enhance ultrasound effects, optimize cellular responses and expand the potential of sonogenetic approaches. [20], [31], [43]

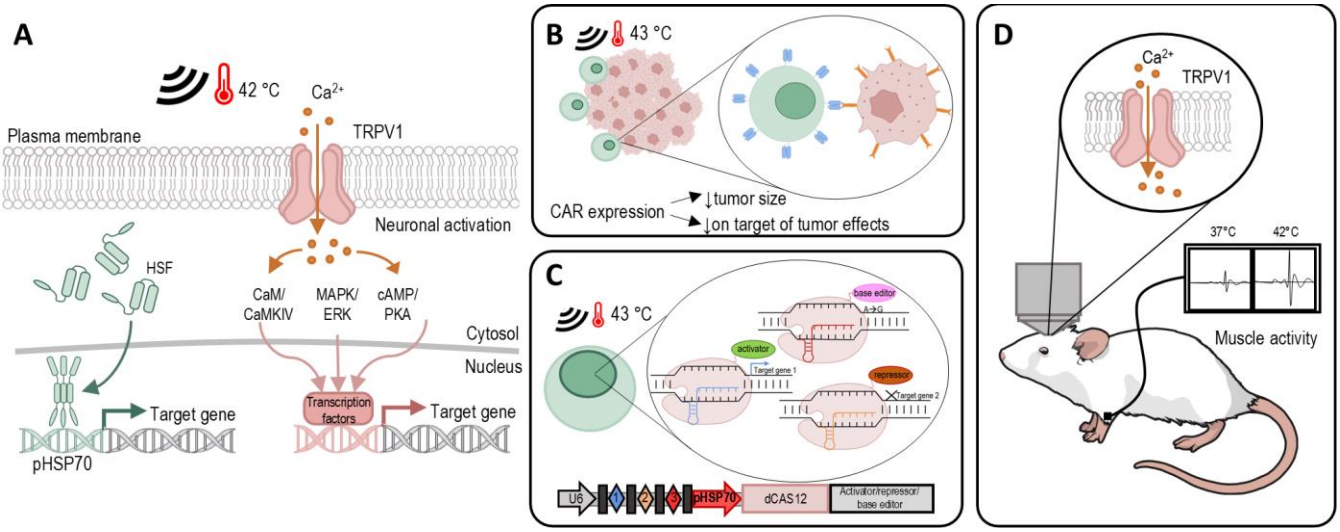

Fig. 2. **Thermosonogenetics for heat-induced gene regulation and cell activation**. (**A**) Focused ultrasound (FUS) increases the local tissue temperature, which activates heat shock factors (HSFs). After phosphorylation, the HSFs translocate to the nucleus where they bind to the heat shock promoter pHSP70 and induce target gene expression. The increase in temperature activates TRPV1 channels in the neurons, leading to Ca^{2+} influx and cellular activation. (**B**) Heat-sensitive promoters have been developed to regulate the expression of chimeric antigen receptors (CARs) in immune cells, enabling spatially and temporally controlled CAR expression. This approach reduces tumor size while minimizing off-target immune responses. [54] (**C**) A heat-inducible CRISPR/dCas12 system enables precise gene regulation, allowing targeted gene activation, repression or base editing in response to thermal stimuli. [57] (**D**) Finally, overexpression of TRPV1 was used to achieve heat-induced neuronal activation in mice. [64]

curve and it outperforms not only the FUS-CAR but also the constitutively expressed CAR when targeting challenging antigens such as GD2, as the EchoBack cells are less exhausted and have a better retention on the cell surface. A 15-minute stimulation at 43°C was required for sufficient expression [55]. Both FUS-CAR and Echoback CAR cells were shown to have a viability of over 90% and no damage due to T cell infiltration [54], [55]. However, the authors did not address damage by heating, as other researchers have found that heating at 43°C for five minutes already leads to variability in fiber diameters without a specific pattern, moderate interstitial edema, and few necrotic fibers with centralized nuclei [52].

Another approach to controlling gene expression is using a heat-inducible dCas9 system coupled with transcriptional repressors or activators to regulate the transcription of native genes [56]. When paired with ultrasound, this system enables precise control of gene expression in deep tissue. It enables a multiplexed gene activation with a single RNA array, further expanding its utility for genome editing applications [57] (**Fig. 2C**). A FUS-enabled genome editing nanodevice (FUGEND) was used in cancer cells, where it induced indel mutations in genes that cause apoptotic resistance [58]. This sensitized the tumors to CAR-T cells and tumor-infiltrating lymphocyte therapies. Recently, FUS-CRISPR technology was used to disrupt telomeres in cancer cells, which increased their susceptibility to CAR-T therapy. In addition, these tumor cells were engineered to express a clinically validated antigen (truncated CD19) upon FUS stimulation so that they could serve as antigen-presenting cells for the activation of synNotch CAR-T cells. This activation led to the release of the transcription factor, production of CARs against a universal tumor antigen (PSMA) only in engaged synNotch CAR-T cells, and the effective elimination of neighboring tumor cells [59].

An alternative approach to the use of heat-sensitive promoters is the activation of temperature-sensitive ion channels. TRP channels, which are known to be activated both mechanically and thermally, have great potential for targeted neuronal activation [60]. Among them, TRPV1 channels are particularly notable for their temperature sensitivity [61]. FUS can be used to selectively activate overexpressed TRPV1 channels in the brain, allowing precise activation of specific neuronal populations [62]. This technique has shown considerable potential for controlling neural circuits and influencing behaviors, such as locomotor activity, with high spatial and temporal precision [63], [64] (**Fig. 2D**). Again, tissue damage must also be considered. The use of higher acoustic pressure, which causes higher temperatures, leads to tissue damage; however, sufficient activation can be achieved at 38.5 °C for 15 minutes, which does not cause tissue damage.

In bacterial systems, circuits based on temperature-sensitive repressors have been introduced [65]. Such bacterial systems have been used for cancer immunotherapy. After FUS stimulation, therapeutic microbes expressed checkpoint inhibitors (CTLA-4, PD-1) [66] or the cytokine IFN-γ, which not only causes apoptosis of cancer cells but also stimulates host macrophages and T-cells [67]. Stimulation was performed at 43 °C for 1 hour or at 45 °C for 30 minutes. Recently, a bacterial system SINGER that allows the expression of therapeutic compounds at a safer temperature of 29 °C has been developed. However, repeated stimulation is needed to achieve therapeutic efficacy [68].

IV. Cavitation Effects

Another effect of ultrasound is cavitation, a phenomenon where microscopic gas bubbles in a liquid rapidly expand and collapse under acoustic pressure. This process generates localized high

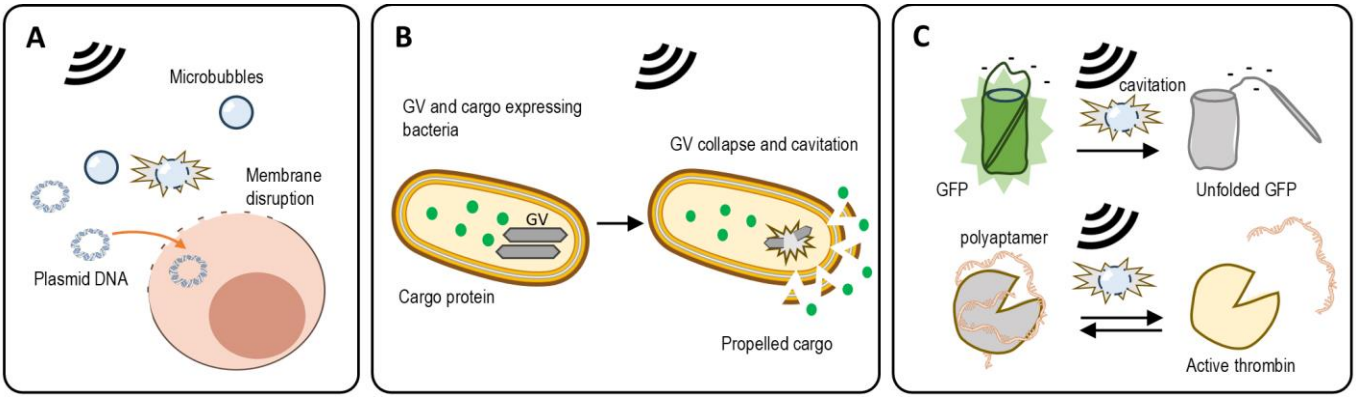

Fig. 3. **Effects and applications of cavitation.** (**A**) Ultrasound-driven microbubbles act as cavitation nuclei that cause transient membrane pore formation. This facilitates the transport of macromolecules such as plasmid DNA, RNA or therapeutic agents into the cells and increases transfection efficiency. [70] (**B**) Gas vesicles (GVs) introduced into bacterial cells serve as genetically controlled cavitation enhancers. Ultrasound-induced collapse of the GVs leads to rupture of the bacterial membrane, releasing intracellular therapeutic cargo for local treatment applications. [75] (**C**) The shear forces generated by cavitation disrupt polymer chains, leading to the controlled unfolding of supercharged molecules such as GFP-SUP. [76] Similarly, thrombin entrapped in polyaptamers is released by ultrasonic stimulation, allowing precise enzymatic activation. [77]

temperatures, microjets, turbulence, shear forces, and shock waves that can disrupt cellular membranes, enhance permeability, and trigger mechanical stress [69].

In biomedical research, cavitation effects at the cellular level are usually coupled with microbubbles, which are then targeted with ultrasound. Membrane disruption with cavitation, termed sonoporation, increases the permeability of the cell membrane to large molecules such as DNA [70], RNA [71], proteins or other polymers into the cells. Thus, sonoporation is an effective method for transfecting mammalian cells (**Fig. 3A**). Sonoporation of immune cells was studied in Jurkat cells, where 563 kPa 1MHz ultrasound rendered 30% of the cells permeable to FITC-labeled dextran [72]. The introduction of genetic material using ultrasound has also been observed in acoustic transfection [73]. The concept of this application is not based on the cavitation of microbubbles but on the disruption of the membrane with high frequencies (150 MHz). Such cross-membrane transport can be useful when Cas protein and guide mRNA are introduced simultaneously [74].

Microbubbles can be replaced by isolated, genetically encoded gas vesicles, which also serve as cavitation nuclei. After their US-induced collapse, the released gas bubble can act similarly to a lipid microbubble. When expressed in bacteria, GV-assisted cavitation can disrupt their membrane and release the cargo previously trapped in the bacteria [75] (**Fig. 3B**).

The shear forces generated by cavitation can stretch and break polymer chains. This principle was applied to develop an ultrasound-responsive protein that contained an additional charged linker. Stimulation of the engineered protein with ultrasound at a frequency of 20 kHz resulted in stretching of the linker and subsequent unfolding and inactivation. By linking an enzyme to its inhibitor, an ultrasound-activated enzyme was produced. [76]. Another approach is the use of long DNA chains produced by rolling circle amplification (RCA), which can act as a polyaptamer that binds enzymes and serves as an inhibitor. Upon stimulation, the polyaptamers break apart and release an active enzyme [77], [78] (**Fig. 3C**). Despite these advances, the use of cavitation in such applications remains challenging due to its potential to damage soft tissues [79]. To address this problem, efforts in this field have focused on optimizing ultrasound parameters with lower mechanical indices [77], [78], [80], [81]. Building on the principle of the RCA, a system of ultrasound-activated proteases coupled with split inteins was developed, allowing the controlled release of biologically relevant molecules, such as hormones, that influence cellular behavior. Sufficient activation was achieved with biocompatible ultrasound that did not affect cell viability [80]. This was further optimized when imaging ultrasound was used to break apart the RCA polymer and release CpG oligonucleotides that activated TLR9 or siRNA that achieved a 75% knockdown. The researchers postulate that since the system's response to the ultrasound stimulation is not facilitated by cavitation, it can be facilitated either by viscous frictional forces between the RCA particles that are fixed in space and the surrounding fluid or by electrostatic repulsion forces between the RCA and the bound oligonucleotide when fluid forces remove counterions.

V. Conclusions

Ultrasound has proven to be a powerful modality for cell modulation as it is non-invasive, penetrates deep into the tissue and allows precise spatial and temporal control. While optogenetics suffers from limited tissue penetration and magnetogenetics requires complex infrastructures, ultrasound offers a unique advantage in the remote control of cellular functions with high precision.

The medical applications of ultrasound are diverse and range from neuromodulation and modulation of immune responses to gene therapy and tissue regeneration. Sonogenetics, in which ultrasound is coupled with genetically engineered effectors, has shown promising results in the activation of mechanosensitive ion channels, the regulation of gene expression and the amplification of cellular responses. Recent innovations such as the use of microbubbles and gas vesicles as acoustic contrast agents have significantly improved the effectiveness of ultrasound in biomedical applications.

Despite its potential, the field faces critical challenges, including the precise identification of ultrasound parameters

(frequency, pressure, duration) that selectively activate, manipulate or influence specific cell types. The development of molecular constructs for the conversion of ultrasound into cellular functions that can be integrated into advanced therapeutic strategies remains an important goal. In addition, inconsistencies in the specification of ultrasound parameters - such as power, pressure, transducer positioning and wave propagation conditions - hinder reproducibility between different studies. The introduction of standardized protocols and reporting guidelines is crucial for the reliable replication and validation of results. The primary technical specifications begin with the details of the ultrasound transducer, including such factors as center frequency, bandwidth, aperture size and shape (e.g., linear, phased, or concave), focal length, and whether the beam is focused. For array transducers, the number of elements and their arrangement must be specified. This information is particularly important if custom transducers are used. In addition, information about the acoustic output must also be documented. This includes the peak negative pressure, mechanical and thermal index (where applicable) and acoustic intensity parameters such as the spatial-peak temporal-average intensity (I_{SPTA}) and spatial-peak pulse-average intensity (I_{SPPA}). The pulse duration, pulse repetition frequency (PRF), duty cycle and total sonication time should be specified, as well as the waveform modulation used, e.g., amplitude or frequency modulation. We believe that a precise distinction between primary parameters and derived parameters is of utmost importance. Primary parameters are those variables that are either directly measured or otherwise determined as part of the experimental setup, and derived parameters are usually calculated from these primary values based on available equations or models. In the context of a report, it is important to report key primary parameters as these allow independent verification, recalculation or reconfiguration of data by other researchers for alternative applications or models. While reporting both parameters with good definitions and units is preferable, priority must be given to providing primary measurements wherever possible. The type of signal — whether continuous wave, pulsed wave or burst mode — should be mentioned as well as the model of the function generator and amplifier used, their settings and any matching networks used. Specifying the control voltage or input voltage of the transducer would be useful, especially for customized transducers. In addition to these technical details, the parameters of the test setup are also important. The orientation and position of the transducer in relation to the tissue/hydrophone should be detailed, including angle of incidence and distance to the surface. The ambient and tissue temperature should be reported, especially if the tissue was temperature-controlled during the experiment including methods used to control or monitor temperature. The calibration methods used for acoustic pressure and intensity measurement—usually a calibrated hydrophone — should be specified, as well as the environmental conditions (e.g., water, degassed water, gel or tissue) and the measurement location (e.g. at the acoustic focus).

Some recent publications address this issue and offer well-written methods that allow for greater reproducibility of results [13], [28], [34], [82].

Future advances should focus on optimizing genetically encoded ultrasound-sensitive constructs, refining acoustic contrast agents and improving the specificity of ultrasound-based therapies. The integration of ultrasound with gene editing tools such as CRISPR and image-guided focused ultrasound (FUS) could further improve its precision and therapeutic applicability.

To summarize, ultrasound-based cell modulation represents a promising future in synthetic biology and medicine. However, to realize its full potential, it is essential to overcome the current technical and standardization challenges. A coordinated effort to standardize experimental methods and refine technological approaches will be crucial to advancing ultrasound as a key tool for innovative biomedical research and therapeutic applications.

Authors' Contributions

FI, VJ and NV drafted the review. RJ and MB revised the manuscript. All authors contributed and approved the submitted version.

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Conflict of Interest Disclosure

The authors declare no competing financial interests.

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