Sonogenetics for non-invasive anti-arrhythmia

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Implantable cardioverter-defibrillator (ICD), a widely used device for severe heart attack patients, is invasive and may cause infection and damage. We propose the use of sonogenetics as a non-invasive alternative. It focuses an ultrasound on stretching cardiac tissue non-invasively, controls stretch-activated channels expressed on cardiomyocytes, and spatiotemporally excites or inhibits cardiac excitation for harmless anti-arrhythmia. Except for traditional antiarrhythmic mechanisms such as inducing activation by a global pulse or local serial pulses like in ICD, the sonogenetics-based anti-arrhythmia has three new mechanisms. This is because, unlike anchoring the tip electrode of ICD in heart chambers, the non-invasive ultrasound can freely change its focus area to create moving line-, ring-, and stairs-shaped electrophysiological patterns on cardiac tissue and to drive arrhythmias away. The intensity and frequency of the ultrasound we use comply with the U.S. Food and Drug Administration standard. The reported results of our in silico experiments can be easily verified by combining existing experimental methods.

Significance Statement
Sonogenetics is a promising method to stimulate excitable cells non-invasively. We first propose to apply it to cardiomyocytes for anti-arrhythmia. It focuses an ultrasound on stretching cardiac tissue non-invasively, controls stretch-activated channels expressed on cardiomyocytes, and spatiotemporally excites or inhibits cardiac excitation for harmless anti-arrhythmia. Thus sonogenetics can be a better treatment of arrhythmias than the traditional but invasive implantable cardioverter-defibrillator.

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Results

In silico experiment. Our research is based on an in silico experiment. It consists of four parts, as shown in Fig. 1: in vitro culture of cardiac tissue, expression of SACs on cardiomyocyte membranes, ultrasonic spatiotemporal selective focusing, and recording of experimental results (see SI, Supplementary text for details). Each part has been implemented in the experiment respectively: in vitro culture of guinea pig and rabbit cardiac tissue (23), expression of Piezo1 channels on neurons (24), ultrasonic phased arrays focusing (25), and fluorescence image recording (26).

Here we suggest using Piezo1 channels as SACs in sonogenetics because of three advantages: 1, The Piezo1 channel is known as the most mechanosensitive channel of SACs (27) which can be activated by low-intensity ultrasound (28) (see SI, Fig. S1); 2, The Piezo1 channel is genetically homologous with humans (29) and can avoid severe immune rejection during transfection; 3, Piezo1 channels may exist in human cardiomyocytes (30), so it is possible to overexpress Piezo1 channels in the human body to replace virus infection.

Results of node dynamics. To verify whether sonogenetics can excite action potential waves on cardiac tissue, we observe the results of node dynamics. We simulate the membrane potential changes at any node in cardiac tissue after applying ultrasonic radiation pressure stimulation. According to the FDA standards, the spatial-peak temporal-average intensity (ISPTA) of medical pulsed ultrasound applied to the heart cannot exceed 430 mW/cm² (31). The ISPTA is calculated by multiplying the ultrasonic intensity $I_0$ by the duration $D$ applied by the ultrasound per second ($ISPTA = I_0D$). The magnitude of the ultrasonic radiation pressure $\Gamma$ is controlled by the ultrasonic intensity $I_0$: $\Gamma = 2I_0/c$ (see SI, Supplementary text), where $c$ is the velocity of ultrasound in cardiac tissue. We calculate the ultrasonic radiation pressure $\Gamma$ under different durations $D$ when $ISPTA = 430$ mW/cm² (see the solid black line in Fig. 2), then calculate the minimum Piezo1 channels’ density $N_{Piezo1}$ required to excite the action potential at the node (see the red dashed line in Fig. 2). When Piezo1 channels’ density exceeds this value, Piezo1 channels’ current generated by ultrasonic radiation pressure will excite the node to generate an action potential (see SI, Fig. S2). During the action potential, cardiomyocytes cannot be excited by an external stimulus. This property is called refractoriness, and this unresponsive period is called the refractory period. Here we use sonogenetics to generate the refractory period to achieve the effect of eliminating spiral waves and turbulence.

Global ultrasound resetting turbulence. Similar to the mechanism of ICD, we first prove that global ultrasound stimulation can reset cardiac electrical signals to eliminate fibrillation. As shown in Fig. 3 (and also in Movie S1), in the beginning, there are turbulence (excited area) and resting areas in the cardiac tissue. Starting from $t = 0$ ms, we apply 1 ms global ultrasound stimulation. At $t = 20$ ms, the whole tissue is excited. Thus the propagation of turbulence is blocked due to refractoriness. At $t = 50$ ms, with the change of action potential, the membrane potentials of all nodes gradually return to resting potential. At $t = 145$ ms, the cardiac tissue is reset to the normal excitable state, and turbulence is eliminated. In this case, according to the requirement for the ISPTA, we use $D=1$ ms and $\Gamma = 41.35$ mmHg.

In addition to the ISPTA, FDA also uses the mechanical index (MI) and the thermal index (TI) to measure the safety of ultrasonic cavitation effect and thermal effect (32). MI is calculated by dividing the peak negative pressure $p$ in MPa by the square root of the ultrasonic frequency in MHz, and the maximum value allowed by the FDA is 1.9. Here we calculate...
the ultrasonic density $I_0$ by the formula $Γ = 2I_0/c$ (note that 1 mmHg=133.32 Pa). Then, substitute $I_0$ into the equation $p = \sqrt{2ρcI_0}$ to calculate $p$, where $c=1561.3$ m/s is the sound velocity in cardiac tissue and $ρ=1081$ kg/m$^3$ is the density of cardiac tissue. TI for soft tissues is defined as the ISPTA in mW/cm$^2$ multiplied by the ultrasound focus area $A$ in cm$^2$ and the ultrasonic frequency in MHz and then divided by 210. The maximum value of TI allowed by the FDA is 6. Thus, the ultrasonic frequency is an important parameter for measuring MI and TI. The minimum ultrasonic frequency satisfying MI is $f_{\text{MIN} \text{MI}}(MHz) = 0.35f(\text{mmHg})/1.9^2$, and the maximum ultrasonic frequency satisfying TI is $f_{\text{MAX} \text{TI}}(MHz) = 2.93/A(cm^2)$. Therefore, we need to find a suitable ultrasonic frequency range (greater than $f_{\text{MIN} \text{MI}}$ and less than $f_{\text{MAX} \text{TI}}$) to ensure the safety of cardiac tissue. In this case of global ultrasound, we calculate that $f_{\text{MIN} \text{MI}} = 4.01$ MHz and $f_{\text{MAX} \text{TI}} = 0.03$ MHz. $f_{\text{MIN} \text{MI}} > f_{\text{MAX} \text{TI}}$, which means that there is no ultrasonic frequency that meets both the FDA requirements for MI and TI. The reason is that the ultrasonic focus area is too large, so the ultrasonic frequency must be very small under safe TI. Therefore we need to shrink the ultrasonic focusing area to reduce TI.

**Ultrasonic strip sweeping off turbulence.** To meet the FDA’s requirements for MI and TI, we have to replace global ultrasonic stimulation with local ultrasonic stimulation. According to the real-time change of the focused area of ultrasonic phased arrays, we use the moving ultrasonic local strip to block turbulence propagation. As shown in Fig. 4 (and also in Movie S2), the ultrasound focuses on the strip area (red shadow). At $t=0$ ms, the ultrasonic strip is located on the left edge of the tissue. Then, it sweeps uniformly from the left side of the tissue to the right until it leaves, as shown in the 50 ms and 150 ms snapshots in Fig. 4. The nodes in the area swept by the ultrasonic strip are excited and become refractory, so turbulence is blocked and gradually disappears. At $t=355$ ms, turbulence has completely disappeared, and the cardiac tissue return to the excitable state. In this case, the strip width is 0.05 cm, the moving speed is 0.05 cm/ms, and the ultrasonic radiation pressure $\Gamma = 51.68$ mmHg. A video of turbulence being swept is shown in Movie S2.

Ultrasound series overdriving the spiral wave. The appearance of spiral waves in the heart is clinically corresponding to tachycardia and may turn into fatal fibrillation. In excitable media such as the heart, waves with slower oscillation frequencies are always driven out of the boundary by faster waves (33).
According to this principle, ICD electrodes are used clinically to generate fast target waves to overdrive spiral waves to treat slow tachycardia, called anti-tachycardia pacing (34). We propose to use ultrasound instead of ICD electrodes to generate high-frequency target waves non-invasively. Fig. 5 (and Movie S3) show ultrasound generates high-frequency target waves (8 Hz) to overdrive the slow spiral wave (6.3 Hz) out of the tissue boundary. We use the Jacobian-determinant method (35) to mark the position of the tip of the spiral wave (white dot in Fig. 5). If the tip leaves the tissue, it can be determined that the spiral wave will disappear. At \( t=0 \) ms, the ultrasound is pulsed on a circular area (red shadow) to generate target waves. Then, the spiral wave is gradually driven to the boundary by the ultrasonic target wave, and the tip also moves to the boundary (as shown in the 300 ms and 600 ms snapshots in Fig. 5). At \( t=730 \) ms, the tip of the spiral wave leaves the tissue, and then the spiral wave gradually disappears. After the spiral wave is eliminated, we stop the ultrasonic target wave stimulation, and the cardiac tissue returns to the excitable state. In this case, according to the requirement for the ISPTA, we use \( D=0.1 \times 8=0.8 \) ms and \( f=51.68 \) mmHg. Then, we calculate that \( f_{\text{min}}^{\text{pul}}=5.01 \) MHz and \( f_{\text{max}}^{\text{pul}}=5.83 \) MHz.

Ultrasonic generation of target waves can avoid some disadvantages of ICD electrodes. According to the eikonal relation of the excitable medium (36), the target wave must have a large enough excitable radius to propagate. Therefore, the electrodes of the ICD must be large enough to propagate the target wave, which may cause vascular damage when implanted. In addition, these electrodes are hard on the surface of the heart and may fall off when the heart beats, leading to treatment failure. Ultrasonic phased arrays can change the size and position of the focus area in real-time and can effectively prevent these shortcomings of ICD electrodes.

Ultrasonic target waves can effectively eliminate slow spiral waves. However, the frequency of ultrasonic target waves has an upper limit, i.e., the frequency of target waves will not increase indefinitely as the frequency of the ultrasonic pulses increases (see SI, Fig. S4). Therefore, the mechanism of overdriving is only suitable for spiral waves with lower frequencies.

**Ultrasonic ring pulling the spiral wave away.** To eliminate spiral waves of any frequency, we propose a new mechanism. In this mechanism, we need to generate a circular refractory period area and do not need a large excitation area to generate target wave excitation. So, we use a circular ring ultrasonic focusing area instead of a circular area. As shown in Fig. 6 (and also in Movie S4), at \( t=0 \) ms, the ultrasonic ring (red ring) is focused to the right of the tip of the spiral wave to generate a ring-shaped refractory area. The spiral wave cannot pass through this refractory area and will revolve around it, which we call pinning. Then, we slowly move the ultrasonic ring out of the tissue boundary, and the pinned spiral wave is pulled out (as shown in the 300 ms, 600 ms, and 840 ms snapshots in Fig. 6). In this case, the ultrasonic ring moves 0.025 cm to the right every 5 ms. In the movement track of the ultrasonic ring, some areas will be stimulated twice by ultrasound. To meet the FDA’s requirement for the ISPTA, we set the ultrasonic pulses to stimulate once every 5 ms, each lasting 0.5 ms. So, the nodes in the tissue are stimulated by ultrasound for a maximum of 1 ms, and the ISPTA is still within the FDA’s requirement. In addition, due to the small area of the ring, there is a wide ultrasonic frequency range (\( f_{\text{min}}^{\text{pul}}=4.01 \) MHz, \( f_{\text{max}}^{\text{pul}}=45.22 \) MHz). The range of ultrasonic ring’s moving velocity and direction selection that can successfully eliminate this spiral wave is shown in SI, Fig. S5.

**Ultrasonic fast-channel stairs leading the spiral wave out.** To eliminate the spiral wave faster, we propose to use spatiotemporally selective ultrasound to generate a fast channel to lead the spiral wave out. Here, we use a stairs-shaped fast channel to reduce the focusing duration of ultrasound at the same position. At \( t=0 \) ms, we add the first stair around the tip of the spiral wave. Then, the ultrasonic area moves 0.05 cm to the right every 13 ms. After each position update, the ultrasound stimulates for 1 ms, and the upper boundary of the ultrasonic stair is on the same ordinate as the tip of the spiral wave. As shown in the 32 ms, 60 ms, and 175 ms snapshots in Fig. 7 (and also in Movie S5), the tip gradually moves out of the tissue boundary along ultrasonic stairs. In this case, the ultrasound stimulation is mobile, and each node is stimulated within 1 ms. The ISPTA meets the requirement of the FDA, and we calculate that \( f_{\text{min}}^{\text{pul}}=4.01 \) MHz and \( f_{\text{max}}^{\text{pul}}=29.30 \) MHz.

The above two mechanisms both eliminate the spiral wave by guiding the tip out. The success of these two mechanisms is due to the spatiotemporally selective stimulation of ultrasound, which cannot be achieved with ICD electrodes.

**Discussion**

**Proof of model independence.** To demonstrate our results are model-independent, we reproduce the main results using the
guinea pig Luo-Rudy I model (see SI, Supplementary text) (37) with MscL channels’ current equation (see SI, Supplementary text) and fixed electrodes on the endocardium for applying invasive ICD, which needs to implant wires through the vein under this pressure changes, we simulate that the cardiac contraction and relaxation based on the experimental data of neonatal rat cardiomyocytes (38) (see SI, Supplementary text). These prove that the mechanisms of eliminating turbulence and spiral waves by sonogenetics apply to different SACs and cardiac models (see SI, Fig. S6). Note that these works are used to verify model independence and do not take into account FDA requirements for ultrasound safety.

**No side effect of stretch-activated ion channels by heartbeats.** Our numerical simulation results show that the large enough ultrasonic radiation pressure can activate SACs, thus changing the excitability of cardiac tissue to eliminate spiral waves and turbulence. However, when the heart beats, cardiomyocytes contract and diastole in sinus rhythm. Could the contractile and diastolic forces of the cardiomyocytes activate overexpressed SACs, resulting in abnormal electrical signals that are harmful to the heart? To answer this question, we estimate the changes in cardiomyocyte membrane pressure during contraction and relaxation based on the experimental data of neonatal rat cardiomyocytes (38) (see SI, Supplementary text). Under this pressure changes, we simulate that the cardiac contraction and relaxation will not affect our results (see SI, Fig. S7).

**Advantages over other defibrillation methods.** Compared to invasive ICD, which needs to implant wires through the vein and fixed electrodes on the endocardium for applying electrical shocks, our sonogenetics-based method could place the ultrasonic phased array outside the patient’s chest and adjust phases of the array to focus ultrasonic waves on selective areas for effective defibrillation. Compared to optogenetic defibrillation (39–44), which could only illuminate the surface of the heart wall, the ultrasound can penetrate it and control the cardiac excitation there. Compared to previous ultrasonic defibrillation (45), our sonogenetics-based method upregulates the density of SACs on the cardiomyocyte membrane, and uses the ultrasonic phased array to enhance the ultrasonic intensity just in a small area, and thus harmlessly terminates arrhythmia under FDA safety standards.

**Conclusion**

We demonstrate competitive advantages and novel mechanisms of the sonogenetics-based anti-arrhythmia approach in a quantitative numerical model. Our in silico experiment results can be easily verified by a combination of existing experimental methods. Our study reveals that sonogenetics will be a non-invasive and harmless treatment for anti-arrhythmia in clinics.

**Materials and Methods**

According to previous sonogenetics experiments (11, 46), the ultrasonic radiation pressure \( \Gamma \) can be described as (see SI, Supplementary text for the formula derivation):

\[
\Gamma = \frac{2I_0}{c},
\]

where \( I_0 \) is the ultrasonic intensity; \( c \) is the velocity of ultrasound in the medium, which is 1561.3 m/s in cardiac tissue. The ultrasonic radiation pressure \( \Gamma \) can be adjusted by changing the ultrasonic intensity \( I_0 \).

To model the transition of Piezo1 channel’s states under different pressures, Lewis et al. proposed a four-state model based on experimental data (47). We assume that the ultrasound radiation pressure \( \Gamma \) is the pressure on the Piezo1 channels in the cardiomyocyte membranes. We modify their model to obtain a four-state model for the Piezo1 channel controlled by ultrasonic radiation pressure \( \Gamma \):

\[
\frac{dO_{\text{Piezo}1}}{dt} = a(\Gamma)C + I_1d + hI_2 - (b + c + g)O_{\text{Piezo}1},
\]

where \( O_{\text{Piezo}1} \), \( C \), \( I_1 \), and \( I_2 \) are the probabilities that the Piezo1 channel is in open, closed and two inactivation states, respectively. The transition rates between these four states are represented by \( a(\Gamma) \), \( b \), \( c \), \( d \), \( e(\Gamma) \), \( f \), \( g \), and \( h \), where \( a(\Gamma) \) and \( e(\Gamma) \) are related to the ultrasonic radiation pressure \( \Gamma \) (see SI, Fig. S1 a). The detailed description and equations of the four-state model for the Piezo1 channel are shown in SI, Supplementary text.

Then, we use the stochastic modeling to describe the current equation of Piezo1 channels:

\[
I_{\text{Piezo}1} = \bar{g}_{\text{Piezo}1}N_{\text{Piezo}1}O_{\text{Piezo}1}(\Gamma(V - E_{\text{Piezo}1})),
\]

where \( \bar{g}_{\text{Piezo}1} \) and \( E_{\text{Piezo}1} \) are the maximal conductance of a Piezo1 channel and the reversal potential of 27.3 pS and 8.8 mV measured in the experiment (48); \( N_{\text{Piezo}1} \) is the Piezo1 channel’s density, which indicates the total number of Piezo1 channels per cm²; \( O_{\text{Piezo}1} \) is the open probability of Piezo1 channels; \( V \) is the membrane potential.

We propose to add Piezo1 channels’ current to a normal cardiac model to obtain a cardiac model regulated by sonogenetics. Here we use the Fenton-Karma three-variable model (49) (see SI, Supplementary text) to simulate the electrophysiological activities of human ventricular tissue. This model consists of three variables: the membrane potential \( V \), a fast ionic gate \( v \), and a slow ionic gate \( w \). The three variables are used to produce three independent phenomenological currents: a fast inward inactivation current \( I_{fi} \), a slow inward current \( I_{si} \), and a fast outward current \( I_{fo} \).
a slow time-independent rectifying outward current $I_{so}$, and a slow inward inactivation current $I_{si}$. We add Piezo1 channels’ current to this model, and the membrane potential equation is as follows:

$$\partial_t V = \nabla(D \nabla V) - [I_{fi}(V, t) + I_{so}(V)] + I_{si}(V, t) + IPiezo1(V, t)]/C_m,$$

where $D = 0.001 \text{cm}^2/\text{ms}$ is the diffusion constant, and $C_m = 1 \mu \text{F}/\text{cm}^2$ is the membrane capacitance. For 2D simulation, no-flux boundary conditions are used. Time evolutions are calculated using an explicit Euler method. The diffusion part in the cardiac model is calculated by the five-point stencil method. The time and space steps are 0.05 ms and 0.025 cm (49).

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1. FX Witkowski, et al., Spatiotemporal evolution of ventricular fibrillation. Nature 392, 78–82 (1998).
2. Al’ Holden, A last wave from the dying heart. Nature 392, 20–21 (1998).
3. A Gafinrik, et al., Preventing ventricular fibrillation by flattening cardiac restitution. Proc. Natl. Acad. Sci. 97, 6061–6066 (2000).
4. Z Qu, G Hu, A Gafinrik, JW Weiss, Nonlinear and stochastic dynamics in the heart. Phys. reports 541, 61–162 (2014).
5. LF Lipka, J Cornellis, Implantable cardioverter-defibrillators. Am. J. Medicine 86, 221 (2001).
6. BJ Maron, et al., Efficacy of implantable cardioverter-defibrillators for the prevention of sudden death in patients with hypertrophic cardiomyopathy. N Engl J Med 325, 365–373 (2000).
7. G Graham-Roe, How’s my heart. Nature 435, 14–16 (2005).
8. GP Walcott, CJ Kilingsworth, RE Ikeda, Do clinically relevant transhoracic defibrillation energies cause myocardial damage and dysfunction? Resuscitation 59, 59–70 (2003).
9. A Verma, BL Wilkoff, Intravascular pacemaker and defibrillator lead extraction: a state-of-the-art review. Hear. Rhythm 1, 739–745 (2004).
10. R Sankaranarayanan, R Viswanathan, DJ Fox, New developments in cardiac resynchronization therapy. Br. J. Hosp. Medicine 74, 503–509 (2013).
11. S Ibsen, A Borg, C Schutt, S Esener, SH Chalasani, Sonogenetics is a non-invasive approach to activating neurons in cannabinoidolcosis. Nat. communications 6, 1–12 (2015).
12. J Ye, et al., Ultrasound control of neural activity through activation of the mechanosensitive channel mscs. Nanowires 18, 4148–4155 (2018).
13. C Rabut, et al., Ultrasound technologies for imaging and modulating neural activity. Neuron 108, 93–110 (2020).
14. YC Lin, et al., Force-induced conformal changes in piezo1. Nature 573, 230–234 (2019).
15. AH Lewis, J Grandi, Mechanical sensitivity of piezo1 ion channels can be tuned by cellular membrane tension. Elife 4, e02088 (2015).
16. J Wu, AH Lewis, J Grandi, Touch, tension, and transduction—the function and regulation of piezo ion channels. Trends biochemical sciences 42, 57–61 (2017).
17. Q Ouyang, HL Swinney, G Li, Transition from spiral to defect-mediated turbulence driven by a doppler instability. Phys. Rev. Lett. 84, 1047 (2000).
18. B Coste, et al., Piezo1 and piezo2 are essential components of distinct mechanically activated cation channels. Science 330, 55–60 (2010).
19. G Chang, RH Spencer, AT Lee, MT Barclay, DC Rees, Structure of the mscl homolog from mycobacterium tuberculosis: a gated mechanosensitive ion channel. Science 282, 2220–2226 (1998).
20. FH Fenton, A Karma, Vortex dynamics in three-dimensional continuous myocardium with fiber rotation: Filament instability and fibrillation. Chaos: An Interdiscip. J. Nonlinear Sci 8, 20–47 (1998).
21. CH Luo, Y Rudy, A model of the ventricular cardiac action potential, depolarization, repolarization, and their interaction. Circ. research 68, 1501–1526 (1991).
22. K Wang, et al., Cardiac tissue slices: preparation, handling, and successful optical mapping. Am. J. Physiol. Circ. Physiol. 308, H1112–H1125 (2015).
23. M Qu, et al., The mechanosensitive ion channel piezo1 significantly mediates in vitro ultrasound stimulation of neurons. Isocline 21, 448–457 (2019).
24. A Marzo, BW Drinkwater, Holographic acoustic tweezers. Proc. Natl. Acad. Sci. 116, 84–89 (2019).
25. Z Jia, et al., Stimulating cardiac muscle by light: cardiac optogenetics by cell delivery. Circ. Arrhythmia Electrophysiol. 4, 753–760 (2011).