The role of ultrasound operation mode for safely interfering in the heart rate

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Abstract — **Diagnostic ultrasound applies low intensity acoustic waves to noninvasively investigate biological tissues. Higher intensities can alter tissue characteristics, and this is of interest for therapeutic ultrasound, when the occurrence of bioeffects is – to a certain extent – desirable for tissue healing. Relative to cardiology, diagnostic ultrasound is well established, whereas there is an unexplored potential for therapeutic applications. Ultrasound is an alternative source of energy that has different characteristics when compared to electrical energy and so its interference in the cardiac activity might be useful for treating arrhythmias. The objective of this study is to investigate the role of operation mode (continuous / pulsed ultrasound) for interfering in the heart rate without damaging the tissue. Nine Sprague-Dawley rats were anesthetized with isoflurane and exposed to high-intensity, 1-MHz ultrasound. One animal was submitted to continuous wave application, which produced thermal damage. Two groups of four animals were submitted to different pulsed schemes (single / variable pulse repetition frequency). Post-therapy values were divided by pre-therapy values, resulting in normalized values for heart rate and respiratory rate. These values were compared between both pulsed schemes, resulting in a statistically significant difference ($p<0.05$) only for the heart rate. When comparing the post-therapy and pre-therapy absolute values within the variable pulse repetition rate group, there was a heart rate drop ($p<0.05$), whereas there was no significant effect on the ejection fraction and end diastolic volume, meaning that no major damage was produced. The insonification scheme used in this study excludes temperature effects, so the observed effect results from nonthermal mechanisms, possibly from radiation force.**

Keywords — **arrhythmia, chronotropic effect, therapeutic ultrasound, ultrasonic stimulation, tissue damage**

I. INTRODUCTION

Cardiovascular diseases typically result in scarring which interferes in the normal electrical conduction of the heart, thus making one prone to developing arrhythmias. The available treatments for arrhythmias are limited by poor efficacy and serious adverse effects [1]. Paradoxically, side effects associated with antiarrhythmic drugs include proarrhythmia, which is the potential to generate new life-threatening arrhythmias. Thus, there has been a shift towards non-pharmacological therapies, including defibrillators and pacemakers [2].

Defibrillators are very useful for terminating life-threatening tachycardias [1]. As for implantable devices, they usually apply shocks to conscious patients, and thus discharges are painful [3]. Moreover, they do not offer complete protection against death from arrhythmia, since they can fail to shock when needed [4].

Artificial pacemakers work to maintain a minimum safe heart rate by delivering appropriately timed electrical impulses to the heart for treating bradyarrhythmia [4, 5]. Therefore, it only speeds up the heart rate. The pacemaker might indirectly decrease fast heart rates via antitachycardia pacing, though. This is done by sending brief bursts of impulses to the heart at a faster pace than the already accelerated ventricular rate. The aim is to depolarize the heart muscle at the right moment, interrupting the abnormal rhythm and thereby halting the tachycardia. Nevertheless, when ventricular tachycardia rates exceed 200 beats per minute, antitachycardia pacing is not beneficial because it can turn a hemodynamically stable tachyarrhythmia into ventricular fibrillation, thus requiring defibrillation shocks. [5, 6].

A concern related to electrical implantable devices is the use of leads required to deliver energy pulses to stimulate the myocardium. Lead failure remain a clinical problem [7] found in all lead models, ranging from older to newer versions [8]. Complications include systemic infection [9], pericarditis [10], myocardial perforation [11], inappropriate shock deliveries [4], potential for proarrhythmia [12], failure to pace, failure to defibrillate, and even death [13].

Ultrasound is an alternative source of energy that has different characteristics when compared to electrical energy and so its interference in the cardiac activity might be useful for treating arrhythmias. Ultrasonic waves are known to interfere in the cardiac activity of turtle [14], dog [15], frog [16], mouse [17], pig [18], and guinea-pig [19].

The objective of this study is to investigate the effects of ultrasound on the cardiac activity. This is done by assessing the effects on the heart resulting from different modes of operation (continuous wave, pulsed wave at a single pulse repetition frequency and pulsed wave at variable pulse

repetition frequencies). The main challenge is to find the optimal set of acoustical parameters able to induce chronotropic effects without damaging the tissue.

II. Methodology

A. Animals

The experimental conditions were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol # 10104). Nine Sprague-Dawley rats were exposed to high-intensity ultrasound, eight of which were exposed to pulsed ultrasound, and the other one was exposed to continuous wave. Animals were anesthetized with 5% isoflurane for induction of inhalation anesthesia using a chamber and then 1.5 - 2% isoflurane for maintenance anesthesia via face mask. The level of anesthesia was monitored by pedal reflex and respiratory rate, as isoflurane is a respiratory depressant. It is relevant to highlight that during isoflurane anaesthesia, myocardial contractility is maintained and cardiac rhythm is stable except for the occasional occurrence of tachycardia [20].

The skin over the thoracic region was shaved and depilated to maximize acoustic transmission. Rats were placed on a temperature-controlled platform in dorsal recumbancy for ultrasonic cardiac exposure. Respiratory rate, heart rate, temperature and ECG monitoring started prior to ultrasound exposure of the heart, continued during exposure, and ended after ultrasound exposure had ceased.

Since there were no signs of major problems to those exposed to the pulsed sequences, animals were allowed to recover. As for the animal exposed to continuous-wave, it was injured due to thermal damage. This one rat was euthanized while under anesthesia, being placed on a CO_2 chamber for 5 minutes. The heart was removed, fixed, and processed for histological evaluation.

B. Study Equipment

Fig. 1 presents the block diagram of the experimental setup. Four ECG electrodes on the rat platform were coated with gel to contact the paws. After anesthesia, the animal was placed on the platform, which was connected to a THM 150 control box. A temperature rectal probe was used. The temperature, heart rate, respiratory rate and ECG data were filtered and displayed on screen. The 1-MHz ultrasound transducer was driven by a function generator (Agilent 33250A) connected to an RF power amplifier (ENI A 150).

Other than presenting the physiological data on the screen, the VisualSonics Vevo 2100 imaging system was used to monitor the heart through B-mode and M-mode ultrasound. M-mode images were acquired before and after ultrasonic stimulation. A post-processing was made by drawing four lines on top of the M-mode image. This is used by the program to calculate cardiac parameters, such as ejection fraction.

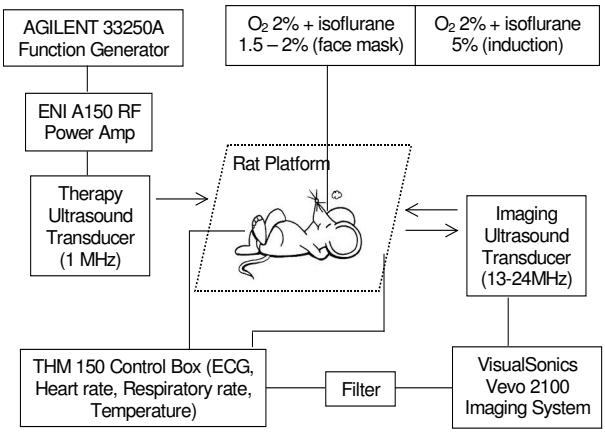


Fig. 1 – Block diagram of the experimental setup, showing ultrasonic stimulation and ultrasonic monitoring.

C. Study Protocol

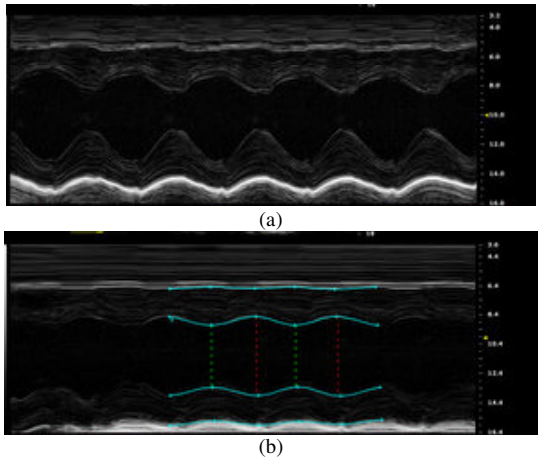
Since the literature describes the use of continuous wave, high-intensity (up to I_{sata}=2.9W/cm^2), 1-MHz ultrasound to produce positive inotropic effects [19], a preliminary experiment was conducted to check for chronotropic effects upon high intensities. When applying a peak negative pressure of 0.6MPa (I_{sata}=1.3W/cm^2) during 1 minute, no effect was observed. However, damage resulted from temperature rise when the heart was exposed to 1.2MPa (I_{sata}=5.2W/cm^2) for 1 minute. In order to minimize thermal effects, two pulsed schemes were proposed: (a) straight pulse repetition frequency, slightly above the heart rate, with variable duty cycles (1%, 5% and 10% for 30s each), at up to 1MPa; (b) duty cycle of approximately 1% with variable pulse repetition frequencies (ranging from slightly above the heart rate until 2 Hz below, for 10s each), at up to 3MPa.

D. Temperature

In addition to the experiments *in vivo*, temperature acquisitions were performed on a tank containing degased, distilled water. The transducer was driven by the same signal generator and RF amplifier used for the cardiac experiments. A heater was used for the water temperature to reach 32^{o}C. A thermocouple (Omega HSTC-TT-T-24S-36.SMPW-CC) was placed 1mm apart from the 1-MHz transducer aperture. The thermocouple was connected to a measurement device (National Instruments USB-TC01) for continuous acquisition, considering that the signal generator started delivering pulses at 10s and stopped at 40s.

III. Results

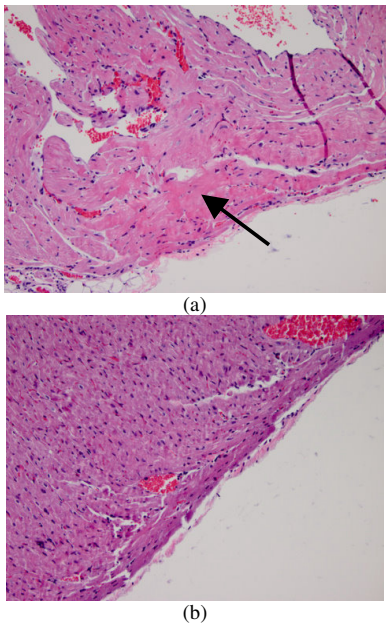
The ejection fraction assessment on top of the M-mode image, as displayed in Fig. 2, indicated a decrease from 84% to 42% after continuous wave ultrasonic stimulation. The histological evaluation shown in Fig. 3 reveals evidence of injury depicted by tissue disorganization and loss of nuclei.



(a)

(b)

Fig. 2 – M-mode (a) before (ejection fraction of 84%) and (b) after (ejection fraction of 42%) continuous wave exposure.



(a)

(b)

Fig. 3 – Histological evaluation magnified 20 times (a) after continuous wave exposure, revealing evidence of damage depicted by tissue disorganization and loss of nuclei (arrow), and (b) after pulsed wave scheme, revealing no damage.

Temperature acquisitions indicated a 5.4°C and a 20°C increases, respectively, when the transducer delivered a peak negative pressure of 0.6MPa and 1.2MPa during continuous exposure (Fig. 4), leading to thermal damage. Fig. 5 shows that the temperature increase caused by a continuous-wave is greater than that caused by a 5% duty cycle burst at a four times higher drive. As for a duty cycle close to 1% at a much higher drive, the temperature increase is negligible (Fig. 6). Thus, for the study to continue at high-intensities but without damaging the tissue, two kinds of pulsed schemes were proposed.

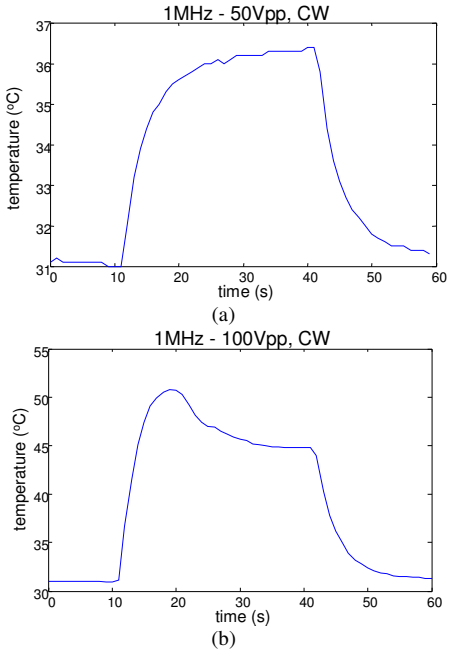


Fig 4: Preliminary continuous-wave application resulted in temperature increases of (a) 5.4°C and (b) 20°C. Continuous-wave combined with high power will result in thermal damage.

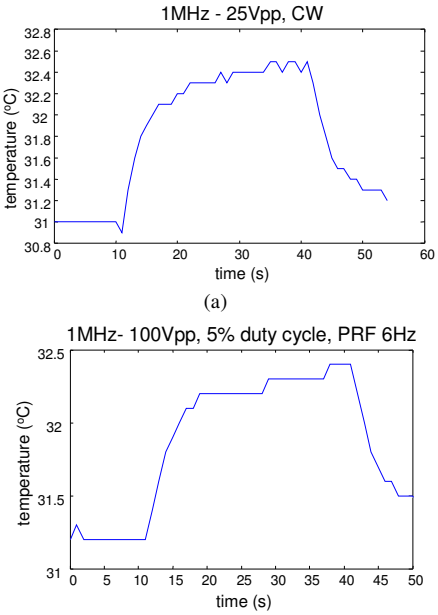


Fig 5: Comparison between the temperature increase caused by (a) a continuous-wave (1.5°C) and (b) a 5% duty cycle burst (single pulse repetition frequency) at a four times higher drive (1.2°C).

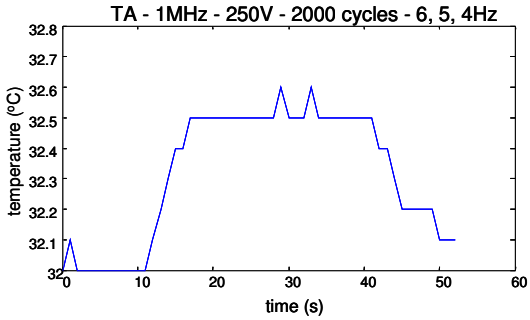


Fig. 6 – Temperature acquisitions when the transducer was driven by $250V_{pp}$ (ouput negative pressure of 3MPa) at about 1% duty cycle and variable pulse repetition frequency. The temperature variation was 0.6°C.

Fig. 7 and Fig. 8 show examples of a heart rate monitoring before and after ultrasonic stimulation with a single pulse repetition frequency and with variable pulse repetition frequencies, correspondingly. In the second case, the heart rate decrease is visible.

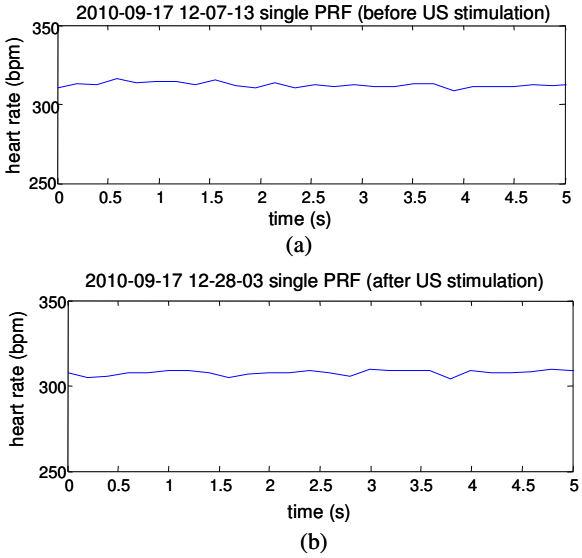


Fig. 7 – Heart rate monitoring (a) before and (b) after ultrasonic stimulation applying a single pulse repetition frequency.

For each pulsed scheme, the values obtained right after the ultrasonic stimulation were normalized by the pre-exposure ones. These numbers are presented as a percentage of the initial values, as shown in Table 1. All values are expressed as mean and standard error of the mean.

Student´s t tests were performed to check whether there were differences in the heart rate and respiratory rate between the two groups, by using the normalized values. Results in Table 2 show a statistically significant ($p<0.05$) heart rate difference, due to a decrease in the variable pulse repetition group. There was no difference between groups for the respiratory rate, which decreased in both cases (see Table 1), probably as a result of isoflurane inhalation. A paired Student´s t test shows a significant ($p<0.05$) heart rate drop whithin the variable pulse repetition frequency group (Table 3).

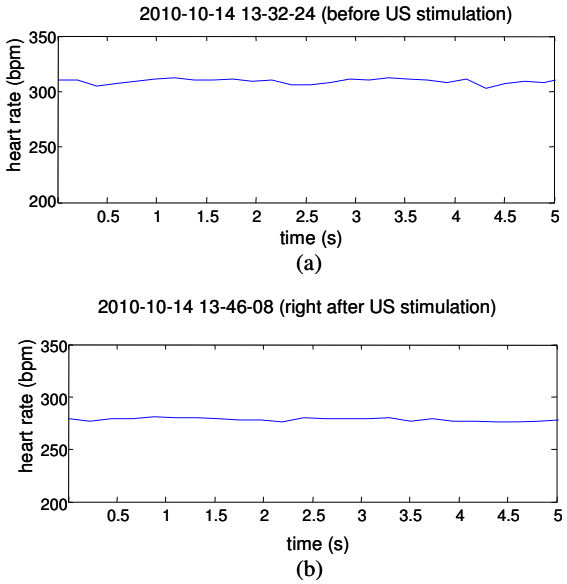


Fig. 8 – Heart rate monitoring (a) before and (b) after ultrasonic stimulation applying variable pulse repetition frequency.

Table 1 – Normalized values of cardiac parameters for each pulsed scheme (single and variable pulse repetition frequency) expressed as mean and standard error (SE) of the mean for n=4.

	Single PRF		Variable PRF	
PARAMETER	MEAN	SE	MEAN	SE
Heart Rate	98.9%	0.26%	81.8%	3.98%
Respiratory Rate	86.5%	6.56%	88.3%	8.56%
Temperature	95.4%	2.54%	96.5%	0.85%

Table 2 – Student´s t test comparing the effects of constant and variable pulse repetition frequencies on heart rate.

Student's t test:Two samples presuming different variances	Heart rate Constant PRF	Heart rate Variable PRF
Mean	0.99	0.82
Variance	0.00	0.01
Observations	4	4
Hypothesis of the population mean difference	0	
Degrees of freedom	3	
t Stat	4.28	
P(T<=t), two tailed	0.02	
two tailed critical t	3.18	

Possible cardiac contractility alterations within the variable pulse repetition frequency group were assessed by means of ejection fraction and end diastolic volume. Such parameters were compared by using the absolute values before and after ultrasonic stimulation to run the paired Student´s t test. No difference was found, as shown in Table 4. Therefore, no major contractile problems occurred.

Table 3 – Paired Student´s t test comparing the heart rate before and after variable pulse repetition exposure. Results show significant difference ($p<0.05$).

Paired Student´s t test for HR	before	after
Mean	333.27	272.09
Variance	327.13	471.683
Observations	4	4
Pearson Correlation	-0.01	
Hypothesis of the population mean difference	0	
Degrees of freedom	3	
t Stat	4.31	
P(T<=t), two tailed	0.02	
two tailed critical t	3.18	

Table 4: Paired Student´s t test comparing the ejection fraction and the end diastolic volume before and after variable pulse repetition exposure. Results show no significant difference ($p>0.05$).

Paired Student´s t test	EF before Variable PRF	EF after Variable PRF	EDV before Variable PRF	EDV after Variable PRF
Mean	82.33	83.41	173.67	182.81
Variance	46.51	40.52	991.31	510.26
Observations	4	4	4	4
Pearson Correlation	0.99		0.86	
Hypothesis of the population mean difference	0		0	
Degrees of freedom	3		3	
t Stat	-2.05		-1.10	
P(T<=t), two tailed	0.13		0.35	
two tailed critical t	3.18		3.18	

IV. Discussion

Back in 1929, Harvey [14] was, perhaps, the first to notice that ultrasonic waves produced a very marked stimulating effect on a beating heart, stating that "the heart muscle is beyond doubt easily stimulated by these waves". At that point, his concern was the difficulty of passing the waves through the thoracic wall. Here, we demonstrated that the 1-MHz ultrasound delivered transthoracically is able to decrease the heart rate by means of a specific sequence of ultrasonic bursts delivered to the heart at a decreasingly variable pulse repetition frequency. The only study in the literature to report ultrasonic pacing [18] did so by submitting the pigs to hypoxia-induced bradycardia, and producing a positive chronotropic effect in order to reestablish the physiological heart rate. The authors applied 3-MPa, 5-ms bursts of 70-kHz ultrasound at a pulse repetition frequency close to the physiological heart rate.

The insonification scheme used in this study excludes temperature effects, so the observed effect results from nonthermal mechanisms, possibly a combination of tissue vibration, promoted by the propagating ultrasonic wave, and radiation force mechanism. The radiation force has been associated with cardiac changes in frogs [16] and pigs [18]. It is a second order effect of the propagating wave which is able to transiently push matter away from the source of ultrasound. In biological tissues, the radiation force is estimated to range from 0.1% to 1% of the instantaneous pressure. High-intensity ultrasound pulses produce greater radiation force effects [21]. Considering the radiation pressure to be 1% of a 3 MPa wave, a transient pressure of 30 kPa (or 0.3 atm or 225 mmHg) would be created on the heart. This is close to the rise in left ventricular intracavitary pressure (250 to 450 mmHg) promoted by a precordial thump [22], which is a single blow that has the potential to promote defibrillation. The precordial thump causes cell membranes to stretch, thus activating ion channels and increasing transmembrane current flow [23].

The heart can be affected by disturbances in its mechanical environment, influencing the origin and spread of cardiac electrical excitation though intra- or extra-cardiac mechanisms. This process is called Mechano-Electric Feedback and it is envolved in many clinical manifestations. It affects the physiological heart rate modulation, the mechanical induction (eg. *commotio cordis*) and termination (eg. precordial thump) of heart rhythm disturbances. This feedback involves mechanosensitive ion channels and mechanical modulation in cellular Ca^{2+} handling [24].

Mechanosensitive channels are able to change open probability in response to a mechanical stimulus [25]. One of these channels is the volume-activated chloride-selective channel (VAC_{Cl}), which is largely distributed throughout the heart and plays a role in arrhythmogenesis, myocardial injury, preconditioning, and apoptosis of myocytes. In spite of its name, VAC_{Cl} is not only stimulated by osmotic and hydrostatic increases in cell volume, but also by direct mechanical stretch [26]. Spontaneously active sino-atrial node cells have shown to reduce their pacemaking rate by approximately 24% via activation of VAC_{Cl} [24]. This effect is similar to the one described here.

Since decreasing the heart rate though parasympathetic stimulation of the heart has been shown to protect against the development of life-threatening arrhythmias [27], the ultrasound sequence proposed here might be protective as well. The cardiac response to ultrasound might be related to that of mechanical stimulation. In this case, ventricular receptors respond decreasing the heart rate due to increased vagal tone. Nonetheless, once a vagal stimulation is

discontinued, effects decay very rapidly [28], which did not occur. In fact, the observed negative chronotropic effect persists after the ultrasonic stimulus is removed. This does not result from the use of isoflurane anesthesia because it does not influence the heart rate in this manner [20]. Thus, in addition to an increased vagal tone, there seems to exist some sort of myocardial conditioning resulting from the ultrasonic stimulation.

V. Conclusion

This is the first study to demonstrate that transthoracic ultrasound can promote a negative chronotropic effect without damaging the cardiac tissue. The 1-MHz ultrasound decreased the heart rate by means of a specific sequence of ultrasonic bursts delivered to the heart at a decreasingly variable pulse repetition frequency. Thus, ultrasound has shown potential to be applied for treating tachyarrhythmias, either exclusively or associated with antitachycardia pacing.

The key to the observed phenomenon seems to be the variation in the pulse repetition frequency, since no chronotropic effect was noticed at constant repetition rates. Moreover, to avoid the risk of producing thermal damage, continuous wave ultrasound at MHz range frequencies should not be combined with high intensities.

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References

[1] B. C. Knollmann and D. M. Roden, "A genetic framework for improving arrhythmia therapy," *Nature*, vol. 451, pp. 929-936, 2008.

[2] S. Nattel, "New ideas about atrial fibrillation 50 years on," *Nature*, vol. 415, pp. 219–226, 2002.

[3] V. Y. Reddy, M. R. Reynolds, P. Neuzil, A. W. Richardson, M. Taborsky, K. Jongnarangsin, S. Kralovec, L. Sediva, J. N. Ruskin, and M. E. Josephson, "Prophylactic catheter ablation for the prevention of defibrillator therapy," *N Engl J Med*, vol. 357, no. 26, pp. 2657-2665, 2007.

[4] D. Graham-Rowe, "Hotwire my heart," *Nature*, vol. 435, pp. 14-15, 2005.

[5] W. Bratsford, "Pacemakers and antitachycardia devices," in *Yale University School of Medicine Heart Book*, B. L. Zaret, M. Moser & L. S. Cohen (1st ed), New York, NY: William Morrow & Co, 1992 (432p).

[6] R. G. Trohman, M. H. Kim, and S. L Pinski, "Cardiac pacing: the state of the art," *Lancet*, vol. 364, pp. 1701–1719, 2004.

[7] K. L. Lee, "In the wireless era: leadless pacing," *Expert Rev Cardiovasc Ther*, vol. 8, no. 2, pp. 171-174, 2010.

[8] T. Kleemann, T. Becker, K. Doenges, M. Vater, J. Senges, S. Schneider, W. Saggau, U. Weisse, and K. Seidl, "Annual rate of transvenous defibrillation lead defects in implantable cardioverter- defibrillators over a period of >10 years," *Circulation*, vol. 115, pp. 2474-2480, 2007.

[9] M. R. Sohail, D. Z. Uslan, A. H. Khan, P. A. Friedman, D. L. Hayes, W. R. Wilson, J. M. Steckelberg, S. Stoner, and L. M. Baddour, "Management and outcome of permanent pacemaker and implantable cardioverterdefibrillator infections," *J Am Coll Cardiol*, vol. 49, pp. 1851-1859, 2007.

[10] K. A. Ellenbogen, M. A. Wood, and R. K. Shepard, "Delayed complications following pacemaker implantation," *Pacing Clin Electrophysiol*, vol. 25, pp. 1155–1158, 2002.

[11] A. K. Bigdeli, A. Beiras-Fernandez, I. Kaczmarek, C. Kowalski, M. Schmoeckel, and B. Reichart, "Successful management of late right ventricular perforation after pacemaker implantation," *Vasc Health Risk Manag*, vol. 6, pp. 27-30, 2010.

[12] R. Tung, P. Zimetbaum, and M. E. Josephson, "A critical appraisal of implantable cardioverter-defibrillator therapy for the prevention of sudden cardiac death," *J Am Coll Cardiol*, vol. 52, pp. 1111-1121, 2008.

[13] W. H. Maisel, "Semper fidelis — consumer protection for patients with implanted medical devices," *N Engl J Med*, vol. 358, pp. 985-987, 2008.

[14] E. N. Harvey, "The effect of high frequency sound waves on heart muscle and other irritable tissues," *Am. J Physiol*, vol. 91, pp. 284–290, 1929.

[15] A. Smailys, Z. Dulevicius, K. Muckus, and K. Dauska, "Investigation of the possibilities of cardiac defibrillation by ultrasound," *Resuscitation*, vol. 9, pp. 233-242, 1981.

[16] D. Dalecki, B. B. Keller, C. H. Raeman, and E. L. Carstensen, "Effects of ultrasound on the frog heart, III: the radiation force mechanism," *Ultrasound Med Biol.*, vol. 23, pp. 275–285, 1997.

[17] A. G. Macrobbie, C. H. Raeman, S. Z. Child, and D. Dalecki, "Thresholds for premature contractions in murine hearts exposed to pulsed ultrasound," *Ultrasound Med Biol*, vol. 23, no. 5, pp. 761 – 765,1997.

[18] B. C. Towe and R. Rho, "Ultrasonic cardiac pacing in the porcine model," *IEEE Trans Biomed Eng*, vol. 53, no. 7, pp. 1446-1448, 2006.

[19] F. Kuma, N. Ueda, H. Ito, T. Maruyama, Y. Kaji, T. Fujino, and M. Harada, "Effects of ultrasound energy application on cardiac performance in open-chest guinea pigs," *Circ J*, vol. 70, no. 10, pp. 1356-1361, 2006.

[20] E. I. Eger 2nd, "The pharmacology of isoflurane, " *Br J Anaesth*, vol 56, no. 1, pp. 71S-99S, 1984.

[21] O. Rudenko, A. Saravazan, and S. Emelianov, "Acoustic radiation force and streaming induced by focused nonlinear ultrasound in a dissipative medium," *J Acoust Soc Am*, vol. 99, no. 5, pp. 2791-2798, 1996.

[22] B. J. Maron and N. A. M. Estes 3rd, "Commotio Cordis," *N Engl J Med*, vol. 362, pp. 917-927, 2010.

[23] M. S. Eisenberg and T. J. Mengert, "Cardiac Resuscitation," *N Engl J Med*, vol. 344, no. 17, pp. 1304-1313, 2001.

[24] P. Kohl and F. Sachs, "Mechano-electric feedback in cardiac cells," *Phil Trans R. Soc London A*, vol. 359, pp. 1173–1185, 2001.

[25] C. X. Gu, P. F. Juranka, and C. E. Morris, "Stretch-activation and stretch-inactivation of shaker-IR, a voltage-gated K1 channel," *Biophys J*, vol. 80, pp. 2678–2693, 2001.

[26] C. M. Baumgarten and H. F. Clemo, "Swelling-activated chloride channels in cardiac physiology and pathophysiology," *Prog Biophys Mol Biol*, vol. 82, pp. 25–42, 2003.

[27] P. J. Schwartz, G. E. Billman, and H. L. Stone, "Autonomic mechanisms in ventricular fibrillation induced by myocardial ischemia during exercise in dogs with healed myocardial infarction. An experimental preparation for sudden cardiac death," *Circulation*, vol. 69, no. 4, pp. 790-800, 1984.

[28] R. M. Berne, M. N. Levy, B. M. Koeppen, and B. A. Stanton, "Physiology", Saint Louis, MO: Mosby, 5th ed, 2003 (1024p).