

Therapeutic Ultrasound in Cardiovascular Medicine

Olivia C. Coiado, PhD , Jacques Lowe, BS, William D. O'Brien Jr, PhD

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Address correspondence to Olivia C. Coiado, PhD, Department of Bioengineering, Carle Illinois College of Medicine, University of Illinois at Urbana-Champaign, 1406 W Green St, Urbana, IL 61801, USA.

E-mail: coiado@illinois.edu

Abbreviations

ASD, atrial septal defect; AVD, aortic valve disease; AVN, atrioventricular node; CW, continuous wave; DF, duty factor; ECWT, extracorporeal shock wave therapy; FDA, Food and Drug Administration; HIFU, high-intensity focused ultrasound; M, maximum temporal intensity; MR, mitral regurgitation; PD, pulse duration; PRF, pulse repetition frequency; PRPA, peak rarefactional pressure amplitude; SATA, spatial-average temporal-average; SPPA, spatial-peak pulse-average; SVT, supraventricular tachycardia; US, ultrasound

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An advantage of therapeutic ultrasound (US) is the ability to cause controlled biological effects noninvasively. Depending on the magnitude and frequency of exposure parameters, US can interact in different ways with a variety of biological tissues. The development and clinical utility of therapeutic US techniques are now rapidly growing, especially with regard to the application of US pulses for cardiac pacing and the potential treatment of cardiovascular diseases. This review outlines the basic principles of US-based therapy in cardiology, including the acoustic properties of the cardiovascular tissue, and the use of US in therapeutic cardiovascular medicine.

Key Words—animal studies; bioeffects; cardiology; cardiovascular diseases; human studies; therapeutic ultrasound

Progress in the application of ultrasound (US)-based medical technologies has grown out of advances in US biophysics, which consider the mechanisms by which US and biological tissues interact with one another.^{1,2} Ultrasound-induced bioeffect studies have focused on US's effects on biomaterials and tissues. Conversely, biological materials' effects on US waves are the basis of US imaging. The scientific basis of risk assessment (bioeffects) and image production (imaging) are provided with the understanding of the interaction of US with tissue, as shown in Figure 1.

The amplitude of the US wave decreases with the penetration distance whenever US energy is propagated into the tissue. The resultant attenuation is due to both scattering and absorption. Absorption represents the part of the US wave that is converted to a temperature increase, whereas scattering is the part of the wave that changes direction. Since the medium can absorb acoustic energy that is converted into heat energy, termed a thermal mechanism, heating may occur if the heat production rate is greater than the heat removal rate. Acoustically generated cavitation is a nonthermal mechanism that has received the most attention, in which US energy is concentrated by cavitation bubbles. Acoustic cavitation broadly refers to bubble activity induced by US in a biological material that contains preexisting gaseous spaces. Mechanisms related to cavitation may include radiation force, microstreaming, shock waves, free radicals, microjets, and strain. Cardiac arrhythmia is a reported bioeffect of myocardial contrast echocardiography, which occurs at relatively high peak rarefactional pressure amplitudes (PRPAs), which are also associated with the destabilization and destruction of the gas body from the US pulses. In particular, US-induced radiation force has received increased attention because of the challenge of

understanding tissues’ mechanical effects, which do not have gas bodies. The described mechanisms of US biophysics, as well as applications, will be discussed in the context of therapeutic US in cardiovascular medicine.

Clinical applications of US in diagnostics are widespread and include obstetrics, gynecology, neurology, cardiology, radiology, oncology, ophthalmology, emergency medicine, and surgery. Diagnostic US is known for providing images of organs and systems to help diagnose medical issues, whereas therapeutic US is used not to produce images but to treat and promote healing. Therapeutic US applications include physical therapy,^{3,4} thrombolysis,⁵ hyperthermia,⁶ lithotripsy,⁷ histotripsy,⁸ ablation therapy,⁹ and promotion of hemostasis.¹⁰ The adoption of US-based therapies in the clinic has benefited from being noninvasive and safe compared with alternative modalities and techniques.¹¹ Additionally, US has a potential cardiovascular application as an alternative energy source for electrical defibrillators, based on the ability of US to produce desired noninvasive biological effects.¹²

Herein, we will review the use of therapeutic US in medicine, outlining the basic biophysical US principles and highlighting studies that suggest US can be used as part of a therapeutic strategy to treat select cardiovascular conditions. An effort has been made to discuss in vitro and in vivo studies in the past 92 years to include a historical overview of therapeutic US within cardiovascular medicine.

The interaction between US and cardiovascular tissue has been studied since the 1920s^{13,14}, when it was discovered that high-frequency sound waves

caused a rhythmic contraction in the ventricular muscles of frogs. During the 1980s and 1990s, therapeutic US began to evolve, and by the 2000s, there were a few tentative uses of US to treat cardiovascular conditions in humans. The techniques and technology developed during this period are indicated in Tables 1 and 2 and discussed below.

Historical Perspective of Ultrasound in Cardiology

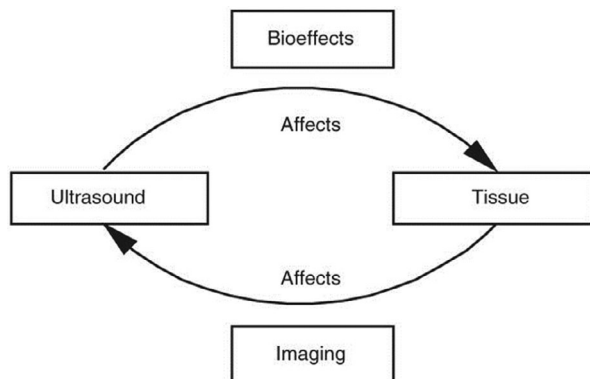
Before 2000: in vitro and in vivo Studies

The mechanism of therapeutic US on the cardiovascular system was first identified by the increase and decrease of zones of pressure in the cardiac tissue, causing a rhythmic contraction in the ventricular muscles¹³ and possible changes in the diastolic force.^{15–17} Studies also suggested that US irradiation might accelerate calcium release or accumulation in the cardiac tissue.^{18–20}

One of the first in vitro studies¹³ used frog’s hearts stimulated by 2 oscillator tubes, a bank of oil condensers, and quartz plates to generate “high-frequency” (continuous wave [CW] 340-kilocycle) sound waves on the heart muscle of frogs and turtles to investigate possible bioeffects. These US waves caused a rhythmic contraction in the ventricular muscles; it was suggested that the effect was not due to temperature changes. It was also considered unlikely that the waves were passing through the thoracic wall because the author speculated that membranes absorbed the waves. Other hypotheses were that the agitation of the medium might be responsible for the stimulation because the heart was sensitive to stretching, which causes contractions. Another explanation provided was the effect of the electrical field, because high-frequency waves were more likely to produce zones of condensations and rarefactions of the medium and locally increase and decrease pressure, thus causing the rhythmic contraction in the heart.¹³

In a more recent study,¹⁵ papillary muscles of rats had exposure to CW 2.3-MHz US in the spatial-average temporal-average (SATA) intensity range of 1.1 to 3.3 W/cm². The experimental setup consisted of a muscle bath, US transducer, and radiofrequency power (Figure 2A). Electrical-stimuli strain gauge

Figure 1. Diagram of US biophysics (modified from O’Brien²).



measures of the papillary muscles were used to assess the US effect on the contractility of the muscles. The strain gauge measures yielded contractile muscle

properties such as the diastolic force or resting force before contraction, the developed force or the maximum systolic force achieved, and the time to peak

Table 1. Time Line of Pertinent Developments Relating to Therapeutic Ultrasound in Cardiology

Development	Study Type	US Type	Study	Year
High-frequency sound waves caused rhythmic contraction in ventricular muscles	In vivo (frog)	US parameters	Harvey	1929
Decrease of the diastolic force in isolated papillary muscle using a frequency of 2.3 MHz	In vitro (rat)	Continuous waves, 1.1–3.3 W/cm ² at 2.3 MHz	Mortimer et al	1980
Cardiac defibrillation caused by US with a frequency of 500 kHz and an intensity of 10 W/cm ²	In vitro (dog)	Continuous waves, 4 W/cm ² at 500 kHz	Smailys et al	1981
Application of 1 MHz in isolated papillary muscle caused a decrease of the diastolic force	In vitro (rat)	Continuous waves, 1 W/cm ² at 1 MHz	Forester et al	1982
Potential of developed force (953 kHz)	In vitro (rat)	Pulsed waves, 1 W/cm ² SATA at 963 kHz	Forester et al	1984
Decrease of the diastolic force, increase of the action potential amplitude	In vitro	Continuous waves, 1.5 W/cm ² SATA at 953 kHz	Mortimer et al	1984
Inotropic effect of CW US related to intensity	In vitro (rat)	Continuous waves, 0.25–2 W/cm ² SATA at 963 kHz	Forester et al	1985
US treatment increased calcium uptake with increasing exposure time	In vitro	Pulsed waves, 0.5 W/cm ² peak, 1 Hz, 2-ms PD	Mortimer and Dyson	1988
Increase in the intracellular concentration of calcium ions	In vitro and in vivo	Continuous waves, 60–480 W/cm ² at 1 MHz	Dinno et al	1989
Arrhythmias	In vitro (rats)	Continuous waves, 3 W/cm ² at 543 kHz	Zakharov et al	1991
Premature ventricular contraction	In vivo (frog)	Continuous waves, 5–10 MPa at 0.7–6 MHz	Dalecki et al	1991
Changes in cardiac rhythm and aortic pressure	In vivo (frog)	Pulsed waves, 390–2.400 W/cm ² peak, 0.5–2 Hz, 5–ms PD	Dalecki et al	1993
Premature contraction of the myocardium	In vivo (mouse)	Pulsed waves, 25–800 W/cm ² peak, 0.5–2 Hz, 5-ms PD	Macrobbie et al	1997
No significant changes in the contraction and a significant effect of US on the stimulation threshold in myocardial cells	In vitro (rats)	Continuous waves, 0.3 MPa at 2.25 MHz	Salz et al	1997
Arrhythmias	In vivo (rats)	Pulsed waves, 15.9 MPa, 1700 Hz PRF, 1.3–μs PD	Zachary et al	2002
Frequency-dependent arrhythmogenic effect	In vitro (rat)	Continuous waves, 0.3 W/cm ² at 45–298 kHz	Petrishchev et al	2003
Positive chronotropic effect	In vivo (guinea pig)	Continuous waves, 2.9 W/cm ² SATA at 1 MHz	Kuma et al	2006
Cardiac pacing	Humans	Pulsed waves, 16.4 W/cm ² at 320–330 KHz	Echt et al	2006
Cardiac pacing	In vivo (pig)	Pulsed waves, 2 kW/cm ² peak, 1.4–2-Hz PRF, 5-ms PD	Towe and Rho	2006
Cardiac pacing	Humans	Pulsed waves, 350 kHz, 0.5-ms PD	Lee et al	2007
Irreversible cardiomyocyte injury	In vivo (rats)	Continuous waves, 1 MPa at 1.7 MHz	Miller et al	2009
Negative chronotropic effect	In vivo (rats)	Pulsed waves, 300 W/cm ² peak, 1 Hz, 2-ms PD	Buiochi et al	2012
Negative chronotropic effect-dependent DF	In vivo (rats)	Pulsed waves, 190 W/cm ² peak, 4–6 Hz, 2-ms PD	Coiado et al	2014
Negative chronotropic effect and the vagus nerve role	In vivo (rats)	Pulsed waves, 190 W/cm ² peak, 4–6 Hz, 167–250-ms PD	Coiado et al	2015
Negative chronotropic effect in different sexes and ages	In vivo (rats)	Pulsed waves, 190 W/cm ² peak, 4–6 Hz, 167–250-ms PD	Coiado et al	2017

Table 2. Summary of Selected Experimental Setups, Including In Vitro, in vivo and Human Studies

Experimental Setup	Study	Year
Frog's heart was stimulated using 2 1-kW oscillator tubes designed for an induction furnace, a bank of oil condensers, and coaxial coils. Quartz plates varying in thickness from 7–14 mm generated waves from 10,000–700,000 cycles or an electric field (50-kV maximum).	Harvey	1929
The muscle bath assembly for suspending and maintaining the papillary muscle is shown in Figure 2A. US transducer consisted of a piezoelectric disk of 13 mm in diameter; the electrical generating system consisted of a signal generator amplified by a radiofrequency power amplifier.	Mortimer et al Forester et al	1980, 1984 1982, 1984, 1985
The experimental arrangement for exposure of cell suspensions to US is shown in Figure 2B. US was generated by a commercial US therapy unit. The exposure chamber was made from thin-wall stainless steel tubing. The exposure vessel was positioned so that the center of the chamber was 100 mm from the face of the transducer. A US-absorbing material was placed at the end of the exposure tank.	Mortimer and Dyson	1988
Pulsed focused US with 543 kHz was applied on rats' hearts isolated by the Langendorff method.	Zakharov et al	1991
0.3-MHz piezoceramic crystals were clamped on the back of a 50-cm-diameter planoconcave lens with a focal length of 54 cm. The crystal bank was charged to 5.5 kV, causing the crystals to expand. To fire the lithotripter, the crystals were shortened to ground. The shorting switch was triggered by a circuit, which sensed the R-wave of the frog's electrocardiogram (Figure 2C).	Dalecki et al	1991
The aorta of frogs was catheterized and coupled to a pressure transducer; high-intensity pulsed US at 1.2 MHz stimulated the myocardial tissue. Signals from the electrocardiogram, pressure transducer, and power to the US were input to a chart recorder and digital oscilloscope for data display and recording.	Dalecki et al Macrobbie et al	1993 1997
The exposure system consisted of 2 vessels: a larger thermostatted tank containing the US transducer, the exposure cell (isolated myocardial cell of adult rats), and a sound absorber. The axis of the US field and the laser beam formed an angle of about 45° to allow the undisturbed measurement of light intensity by a charge-coupled device camera. The exposure cell could be moved in the x-y direction to position the myocyte of interest in the laser beam.	Salz et al	1997
The low-power pulse-echo capability of the exposure system displayed on a digital oscilloscope was used to adjust the calibrated transducer's focal region center 6 mm posterior to the skin surface echo. Fine tuning of the transducer's position was then done until 3 distinct echoes were seen within the focal region. The US beam axis was approximately perpendicular to the heart at the position of the black dot, with the beam's focal region within the heart. The oscilloscope's echo signals were also used to visually determine whether the US field interacted with the contrast agent within the circulatory system during exposure.	Zachary et al	2002
Langendorff perfusion setup and US generator.	Petrishchev et al	2003
US generator system (Figure 3A).	Kuma et al	2006
A steerable bipolar electrophysiology catheter incorporating a receiver electrode into the tip and circuitry to convert US energy to electrical energy was inserted transvenously into the heart. A US-transmitting transducer was placed on the chest wall with US gel. The output waveform of the receiver electrode was monitored while the transmitter was moved on the chest wall to target the receiver. US-	Echt et al Lee et al	2006 2007

(Continues)

Table 2. Continued

Experimental Setup	Study	Year
mediated pacing with minimum voltage but consistent capture was obtained for 12 s (Figure 3C).		
The transducer element was driven by a custom-built field effect transistor power amplifier using a ferrite core step-up transformer that provided up to 4 kV of excitation corresponding to a peak electrical power.	Towe and Rho	2006
The electrocardiographic signal was amplified and sent to an oscilloscope and to a digitizer. The digitized electrocardiogram was analyzed with the aid of software, which provided automated collection of data on the heart rate and the numbers of normal complexes. The software also partially automated detection of premature complexes.	Miller et al	2009
A 1–3.5-MHz US transducer was driven by a function generator connected to a radiofrequency power amplifier. The rat's heart was exposed to pulsed US, and the cardiac parameters were monitored with imaging US (Figure 3B).	Buiochi et al Coiado et al	2012 2014, 2015, 2017

force or the period between the stimulation of the muscle and the maximum rate of rising systolic force (dF/dt). After mechanical parameters and temperature measurements were obtained, a proportional increase of the bath's ambient temperature was performed. The contractile properties of cardiac muscle were affected by temperature. The comparison between the US responses versus the thermal equivalent response showed that both equivalent changes in temperature and US lead to a significant decrease of both the time to peak force and developed force. A decrease of diastolic tension was observed compared with the equivalent thermal intervention.¹⁵ An *in vitro* study¹⁹ using a US therapy unit exposed fibroblasts cells to pulsed waves at 1 Hz, and the US treatment increased calcium uptake with increasing exposure time (Figure 2B).

In a subsequent study,²¹ CW 500-kHz, 10-W/cm² SATA intensity US yielded a cardiac defibrillation and antiarrhythmic effect in the heart of dogs. The antiarrhythmic effect of US can be generated with a lower intensity for the ventricular myocardium than that required for the heart as a whole. The effect can be explained by the hypothesis that the US waves are partially absorbed and partially dispersed by the large mass of the myocardium, resulting in an inhibition of the electrical activity and a decrease of its refractory period of the cells of the myocardium.

In vitro and *in vivo* studies in subsequent years found that the decrease of the diastolic force is a

recurrent US effect.^{16,17} With CW 1-MHz, 1-W/cm² SATA intensity US, a decrease of diastolic force was observed in rat hearts without simultaneous modification of the developed force. These changes were attributed to nonthermal effects because temperature changes were not observed. It was also speculated that US accelerated a release or accumulation of calcium.¹⁶ The effect of US exposure (4 seconds, 1.0 W/cm² SATA at 963 kHz) on the post-tetanic potentiation of isolated isometrically contracting papillary muscle of rats caused a potentiation of the developed force. This showed an important connection between contracting cardiac muscles and short-term CW US exposure. Another study²² showed that the release and transport of calcium were highly likely to be involved in the potentiation of the developed force. Ultrasound (CW 1 MHz, 1.5 W/cm² SATA) caused simultaneous alterations in isolated cardiac muscle.¹⁷ In a previous study,¹⁵ a decrease of diastolic tension had been observed, whereas in this later study, an increase of the action potential amplitude was reported in addition to the decrease of the diastolic force. Ultrasound (CW 1 MHz, 0.25–2 W/cm² SATA) was applied to the rat isolated papillary muscle, and after US stimulation, a positive inotropic effect was observed to be linearly related to the US intensity.¹⁸ Therapeutic-level CW US^{16,22} was reported to increase the force and enhance the effect of graded intensities on rat isolated papillary muscle contractile performance. Based on studies by Mortimer et al^{15,17}

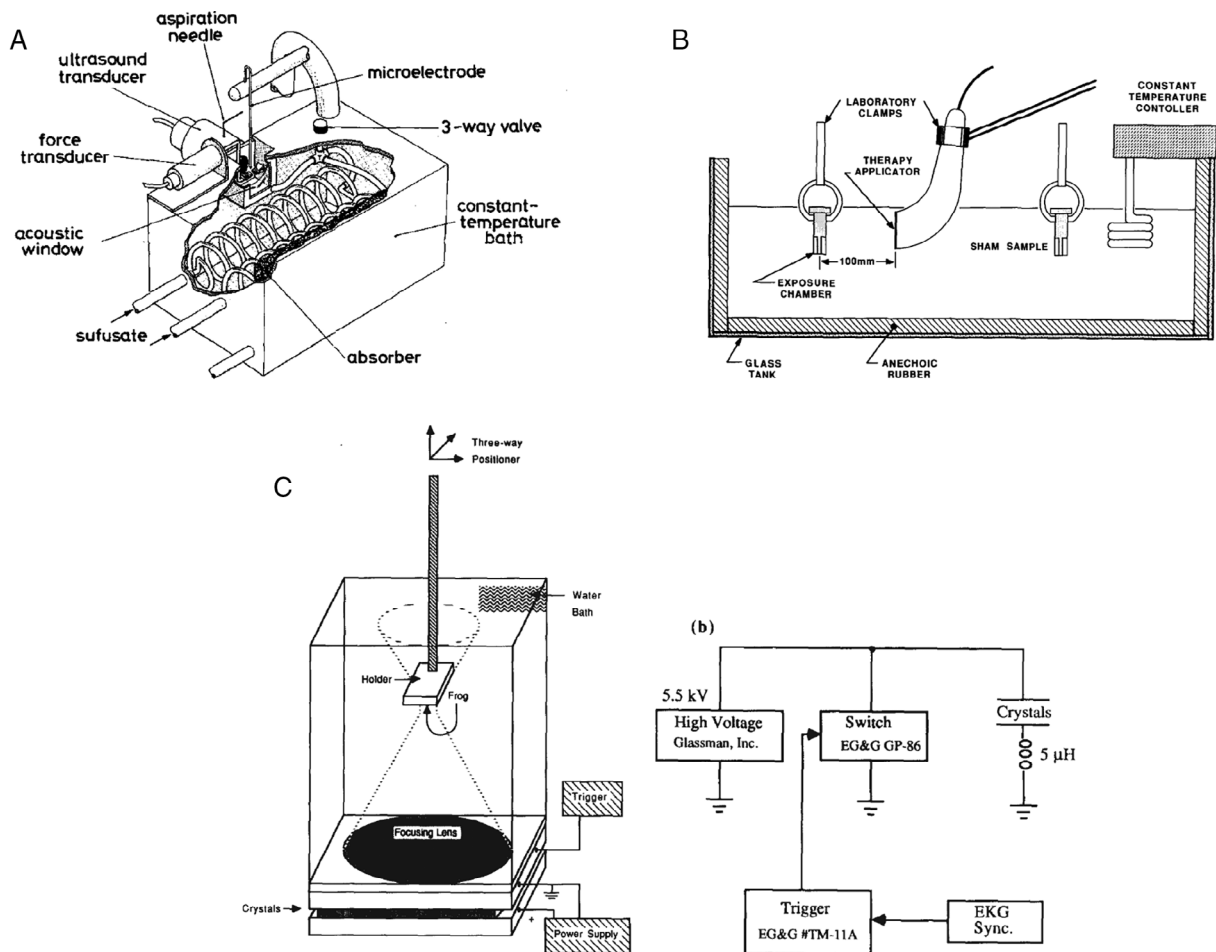
and Forester et al,^{16,18,22} the US stimulation might accelerate a release or accumulation of calcium in the cardiac tissue, and the effect mechanism was viewed to be with nonthermal effects.

More recent reports demonstrate that US application can reduce the threshold for cardiac electrical excitation²³ and produce positive inotropic effects in isolated myocardial preparations by increasing the influx of calcium into cardiac cells.^{12,24} In an in vivo study in frogs²⁵ (Figure 2C), high-intensity US pulses (1.2 MHz, up to 2000 W/cm² spatial-peak pulse-average [SPPA]) caused changes in the heart rate but did not demonstrate effects because the rhythm of the heart and aortic pressure returned to normal

shortly after exposure ceased. Studies in isolated perfused hearts of rats with physiologic saline showed that acoustic cavitation was followed by a decrease of the developed pressure, and no US effects below the same acoustic cavitation intensity were found.^{26,27}

Vykhodtseva et al²⁸ performed an investigation into the effects of high-intensity pulsed US (1.2 MHz, up to 2000 W/cm² SPPA) for the treatment of brain disorders. The multiple pulsed experiments in rabbit brain demonstrated that the histologic effects varied from zero visual damage of tissue to local hemorrhage. As the pulse duration (PD), number of pulses, and repetition frequency increased, the severity of the tissue damage increased. Another in vivo study²⁹

Figure 2. In vitro experimental setups. **A.** Papillary muscle exposed at 953-kHz continuous waves (modified from Mortimer et al¹⁷). **B.** Cells exposed at 1-Hz pulsed waves (modified from Mortimer and Dyson¹⁹). **C.** Frogs' heart exposed at 0.5- to 2-Hz pulsed waves (modified from Dalecki et al⁴⁹).



showed that free gas bubbles were induced in living mammalian tissue by 0.75-MHz US irradiation at 680 mW/cm²; however, the study did not show histologic results. There were no effects of US below the cavitation intensity found. The importance of this work is to explain the bioeffects involved in cardiac pacing during US exposure and find a therapeutic application for cardiac conditions.

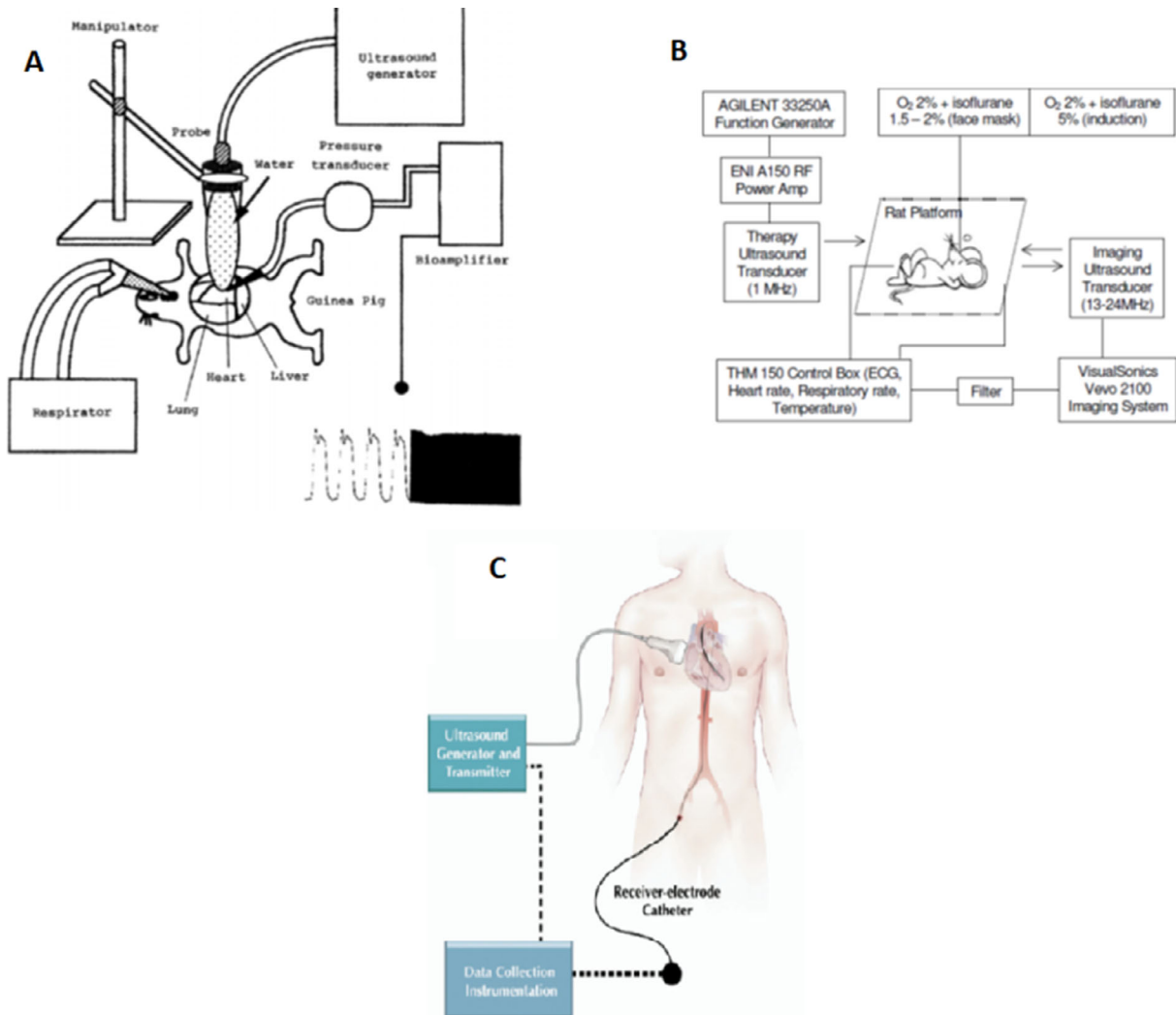
After 2000: in vitro and in vivo Studies

Studies after 2000 suggested that the decrease of the heart rate effect caused by US application on the

heart of rats likely resulted from parasympathetic stimulation or direct mechanical US stimulation of aortic baroreceptors with consequent stimulation of the baroreceptor reflex.^{30,31} In a porcine model, the combination of a radiation force mechanism and tissue vibration was suggested as a possible cause of the US cardiac pacing.³²

Arrhythmia occurred coincidentally with diagnostic US exposures and ceased after US exposures stopped.³³ The heart abnormalities were induced principally when the contrast agent interacted with US during application (pulsed 3.1-MHz frequency,

Figure 3. In vivo and human experimental setups. **A.** Positive chronotropic effect observed in guinea pigs (modified from Kuma et al⁵¹). **B.** Negative chronotropic effect observed in rats (Modified from Buiochi et al³⁰). **C.** Cardiac pacing in humans (modified from Echt et al⁴²).



1.3-millisecond PD, 1700-Hz pulse repetition frequency [PRF], and 15.9-MPa PRPA), and the study suggested that US pulses may have the potential to cause arrhythmias via their biomechanical interactions with contrast agents.³⁴

Transthoracic cardiac US stimulation (pulsed 1 MHz, 3-MPa PRPA) at an approximately 1% duty factor (DF) induced a negative chronotropic effect in rat hearts without damage to the hemodynamic system.³⁰ A likely mechanism to explain the negative chronotropic response to pulsed US exposure would be reflex vagal activation and sympathetic inhibition involving the baroreceptor reflex (eg, by direct mechanical stimulation of aortic baroreceptors by US) or the Bezold-Jarisch reflex. This reflex can be followed by apnea, bradycardia, and hypotension, depends on intact vagi, and is mediated through cranial nervous medullary centers controlling respiration, heart rate, and vasomotor tone.

Another *in vivo* study³¹ showed a negative chronotropic effect in rat hearts (pulsed 3.5 MHz, 2-MPa PRPA [$\approx 133\text{-W/cm}^2$ SPPA], 0.25%–1.0% DF, 2-millisecond PD, 4–5-Hz PRF) showed that the DF more likely influenced cardiac pacing than the pulsed pressure amplitudes. Another bioeffect of myocardial contrast echocardiography is cardiac arrhythmia, which occurs at high PRPAs that are correlated with destruction and gas body destabilization caused by the US pulses. Experiments in perfused rat hearts demonstrated that US (1 MHz, 3-MPa PRPA [300-W/cm^2 SPPA], 1% DF, 5-millisecond PD, 2-Hz PRF) exerted a markedly arrhythmogenic effect on the heart, which, in addition to a mild negative chronotropic effect, might cause a deleterious influence on blood pumping.³⁵

The study of cardiac pacing using US (313–385 kHz, 22.7-W/cm^2 mean PRPA [$0.74\text{--}112\text{-W/cm}^2$ SPTA], mechanical index <1.9, 0.5-millisecond PD) is relevant as a replaceable source of energy to substitute electrical power in implantable pacemakers. Pacing leads are often associated with complications such as dislodgement, infection, and fracture, so there is a medical need to develop a system that could pace the heart and reduce the particular problems caused by pacing leads.³⁶ The removal of a failed lead that has been implanted for a long time could be a high-risk procedure, potentially causing death or arterial injuries.^{37–39} Experiments *in vitro* in rat hearts demonstrated that the US exerted a

markedly arrhythmogenic effect and a negative chronotropic effect.³⁵ In the future, the rat model will likely be translated to large animals such as pigs or dogs to evaluate the feasibility and safety of therapeutic US. The major contribution of this research will be to improve treatment with pacemakers.

In vivo pulsed experiments (1 MHz, 2–3-MPa PRPA [$\approx 133\text{--}300\text{-W/cm}^2$ SPPA], $\approx 1.0\%$ DF, 2-millisecond PD, 4–6-Hz PRF) also have demonstrated that US applied to the chest has the potential to cause a negative chronotropic effect without damage to the cardiac tissue.^{30,40,41} To study this effect, a bilateral vagotomy was performed, with a small vertical midline incision 1 cm superior to the sternum of the rat, to minimize the time the carotid artery was cannulated for the measurement of arterial pressure.⁴⁰ It was hypothesized that the negative chronotropic effect was a direct mechanism caused by US pulses, and the parasympathetic nervous system did not play a role.

Translation to Human Studies

In medicine, US has been used in both diagnosis and disease treatment. Ongoing medical research on US has stimulated the improvement of existing techniques and development of new applications.^{30,40–43} Echt et al⁴² investigated the use of US as an alternative source of energy for pacing without leads (Figure 3C). One potential research application is sex differences not only in cardiology but also in other specialties.⁴⁴ In the United States, deaths due to cardiovascular disease in women exceed those in men, and cardiovascular disease remains the primary cause of death worldwide. Physiologic differences between women and men and cultural factors can contribute to the development of cardiovascular disease.³³ Of note, the incidence and the increased rate of cardiovascular disease are markedly higher in age-matched premenopausal women than men.⁴⁵ The ovarian hormones may be important in reducing the risk of vascular disease in women, as they cause a delay in the onset of vascular disease compared to men. There is a possible temporal link between menopause and the rise in vascular events.⁴⁶ The lack of ovarian hormones is associated with a greater rate of cardiovascular disease, and women with hysterectomy have a higher prevalence and incidence of cardiovascular disease and hypertension.⁴⁷ The hypothesis that female

sex and/or the sex hormone estrogen may contribute to the sexual dimorphism in the heart and to a better outcome of cardiac diseases in women is supported by cardiovascular disease animal models. In a recent study, the role of age and sex in the decrease of the heart rate was investigated with exposure of the rat heart to 3.5-MHz pulsed US.⁴⁸ The study showed a negative chronotropic effect caused by pulsed US, and ovarian hormones were responsible for different US-induced cardiac bioeffects.

Over the years, various US exposure models have been developed. Pulsed US has been shown to interfere in the cardiac activity of the turtle,¹³ dog,²¹ frog,⁴⁹ mouse,⁵⁰ pig,³² guinea pig,⁵¹ and rat^{30,34,35,40,41}. Kuma et al⁵¹ showed a positive chronotropic effect in hearts of guinea pigs (Figure 3A), whereas Coiado et al³¹ observed a negative chronotropic effect in hearts of rats (Figure 3B). Animal models have been developed for different kinds of research, specifically models to identify sex-related differences, including brain injury, atherosclerosis, toxicology, autoimmune diseases, hormones, and stress/alcohol consumption.⁵² Coiado and O'Brien⁴¹ investigated whether sex differences could affect the outcomes in cardiac US therapy. The study showed the feasibility and biophysics of a new technology that uses US pulses to achieve cardiac leadless pacing without causing undesirable side effects. To overcome the limitation of pacemaker leads, a new technology that used pulsed US at a mean frequency of 350 kHz and an 0.5-millisecond PD to achieve cardiac pacing was considered the first human demonstration of cardiac stimulation.³⁶ The safety and feasibility of cardiac stimulation using an alternative energy source proved to be feasible and safe.^{36,42}

Thermal and Nonthermal Mechanisms

Thermal

The amplitude of the US wave decreases with distance whenever it propagates into tissue or any attenuating material; this attenuation is due to absorption and scattering. Scattering can be defined as a portion of the wave that changes direction, and absorption is a mechanism that represents that portion of the wave energy that is converted into an increase of temperature (heat).

The thermal mechanism is relatively well studied. The increase of temperature produced by US is well

known by mathematical modeling techniques^{53–62} and has been estimated for various exposure conditions.^{63,64}

The US wave propagation transports and dissipates energy; the average energy density is

$$\langle E \rangle = \frac{1}{T} \int_0^T E(x, t) dt = \frac{\rho}{2} (U_{op}^2 + U_{on}^2). \quad (1)$$

The instantaneous intensity is defined as the dot product of the US pressure and particle velocity, but because these 2 quantities are in phase, the dot product is pu . Its temporal average representation is given by

$$I = \frac{1}{T} \int_0^T pudt = \frac{\rho c}{2} (U_{op}^2 - U_{on}^2), \quad (2)$$

where U_{op} and U_{on} are the particle velocity amplitudes for the positive and the negative directed components. For a progressive US plane wave propagating in only the positive x direction, $U_{on}^2 = 0$, for standing waves $U_{op}^2 = U_{on}^2$, then $U_{op}^2 = U_o^2$:

$$\langle E \rangle = \frac{\rho}{2} U_o^2 = \frac{1}{2\rho c^2} p_o^2 \quad (3)$$

and

$$I = \frac{\rho}{c} U_o^2 = \frac{1}{2\rho c} p_o^2 = \frac{p_o U_o}{2} \quad (4)$$

In tissue, at the site (spatial peak) where the US spatial-peak temporal-average intensity is I_{TA} , the rate of heat generation per unit volume is given by the expression^{55,57}

$$\dot{Q} = 2\alpha I_{TA} = \frac{app^*}{\rho c}, \quad (5)$$

where

$$I_{TA} = \frac{pp^*}{2\rho c}, \quad (6)$$

where α is the US amplitude absorption coefficient, which increases with increasing frequency; p and p^* are

the instantaneous US pressure and its complex conjugate, respectively; ρ is density; and c is sound speed. The product of p and p^* is equal to the US pressure amplitude squared, p_0^2 , at the specific location in the medium where \dot{Q} is determined and can be thought of as a spatial-peak temporal-average quantity:

$$\dot{Q} = \frac{\alpha p_0^2}{\rho c}. \tag{7}$$

For a given I_{TA} , the maximum temperature increase, ΔT_{max} under the assumption that no heat is lost by convection, conduction, or any other processes to remove heat, is described approximately below⁶⁵:

$$\Delta T_{max} = \frac{\dot{Q} \Delta t}{C_v}, \tag{8}$$

where Δt is the time duration (or also the PD of a single pulse) of exposure, and C_v is the tissue's heat capacity per unit volume. Equation 8 is valid exclusively for short exposure times (<1 minute); for longer exposure times, heat removal processes become significant. As an estimate, we can calculate ΔT_{max} using the US cardiac exposures reported by Buiocchi et al,³⁰ for which the peak pulse pressure amplitude was 3 MPa with a 1% DF. These exposure quantities yield a pulse-average intensity (intensity averaged over only the PD) of 300 W/cm² and a time-average intensity of 3 W/cm², and at an US frequency of 1 MHz, respectively, yields $\dot{Q} = 30$ and 0.3 J/cm³-s ($\alpha \approx 0.05/\text{cm}$ at 1 MHz). Since biological tissues' thermal properties can be approximated by water ($C_v = 4.18 \text{ J/cm}^3 \cdot ^\circ\text{C}$), the maximum rates of change of temperature from Equation 8 are, respectively,

$$\frac{\Delta T_{max}}{\Delta t} = 7.2 \text{ and } 0.072 \frac{^\circ\text{C}}{\text{s}}. \tag{9}$$

Thus, for a 200-millisecond PD (maximum temperature increase caused by a single 200-millisecond pulse), ΔT_{max} would be about 1.4°C, and for a 2-second exposure duration (time between the onset of 2 consecutive pulses), it would be about 0.144°C. The difference between these 2 ΔT_{max} calculations is

that the latter one includes a cooling duration of 2–0.2 seconds = 1.8 seconds.

Nonthermal

Both first- and second-order US quantities have been involved in nonthermally produced biological effects.⁶⁶ Acoustically generated cavitation is the non-thermal mechanism that has received the most attention, principally from US contrast agent microbubbles. Outstanding literature reviews of cavitation have been published.^{54,63,64,66–78}

Concerns have been addressed regarding the interaction of US with contrast agents in humans and potential bioeffects of inertial cavitation.^{66,76} Some studies raised these concerns by documenting the hemolysis of erythrocytes in cell suspensions in human and in mice that contained contrast agents that were exposed to pulsed US.^{79–85} Hemorrhage in the vascular beds of the intestine and skin^{86,87} plus damage to cells in the heart⁸⁸ were also studied in dogs and mice, respectively, after exposure to pulsed US and intravenous injection of a contrast agent. *in vivo* studies have shown induction of hemolysis and petechiae,^{87–92} damage to the intestinal wall,^{81–83,93,94} as well as modification of the blood–brain barrier.^{95,96}

Medical reports of arrhythmogenic changes showed that triggered second-harmonic imaging of a US contrast agent for myocardial perfusion caused premature ventricular contractions in healthy adults⁹⁷ Another report showed arrhythmogenic changes in patients at risk for supraventricular tachycardia, syncope, or ventricular tachycardia caused by non-sustained ventricular tachycardia after intravenous administration of a US contrast agent and exposure to low-frequency therapeutic US.⁹⁷ It has been suggested that cavitation is the mechanism likely for microbubble-induced premature contractions in the heart.⁹⁸

Several diagnostic US techniques using static or dynamic acoustic radiation force have emerged; these techniques are able to locally vibrate tissue, including acoustic radiation force impulse imaging,⁹⁹ vibro-acoustography,^{100,101} and supersonic shear imaging.¹⁰² In general, these techniques use the US-induced temporal-average force on the medium to initiate a biophysical effect. The effect's magnitude is proportional to the local temporal-average intensity.

Whether there are other biophysical effects such as permanent or temporary risk-related tissue responses has yet to be studied extensively.

However, US-induced temporal-average force has been a mechanism implicated in the association with the tactile response^{103–107} and cardiac changes in frogs^{25,108} and pigs.³² The radiation force in biological tissues is estimated to range from 0.1% to 1% of the instantaneous US pressure amplitude. Considering a radiation pressure of 1% of a 3-MPa peak pressure amplitude wave, a transient pressure of 30 kPa (0.3 atm) would be created on the heart. This transient increase of pressure is similar to the occurrence described during a precordial thump, a single blow that has the potential to promote defibrillation.¹⁰⁹

Discussion and Conclusions

In cardiovascular medicine, the pulsed wave velocity and a pressure myograph are commonly used to measure the elastic properties of blood vessels.¹¹¹ Scanning acoustic microscopy has also been used as a method for mapping mechanical properties of isolated cells and tissues.¹¹⁰ Some studies that used these techniques showed increased stiffness of cardiomyocyte cells with age.^{111,112} However, these techniques were not used in the reviewed studies to explore the possible mechanical properties of the cardiac tissue after US application.

Coiado and O'Brien⁴¹ observed a negative chronotropic effect in young female rats; one of the hypotheses is that the US effect is weight dependent. It may be possible that US field interacts with more cardiac structures in smaller animals than in larger animals. However, it is not clear what biomechanism is involved in the negative chronotropic effect.⁴¹ The authors hypothesized that the decrease of the heart rate could be a direct or indirect US (mechanical) stimulation of aortic baroreceptors that can cause bradycardia. It is well known that the velocity of sound in bone is different from that in other tissues. In some cardiac therapeutic studies,^{30–31,36,40–43} the US was used as an external and alternative source of energy to pace the heart. Lee et al³⁶ showed a refractive effect from the ribs, with attenuation and absorption during US stimulation. To minimize these mechanical effects, the heart was exposed to different amplitudes

of US energy during pacing attempts. The authors mentioned in this review that more studies are necessary to elucidate the mechanisms of muscle contractility, including beat-beat variability and the negative chronotropic effect.

Diagnostic US operates on the hypothesized premise that it is noninvasive, low cost, and safe compared with other diagnostic imaging modalities. In the mid-1970s the safety of US and the regulatory parameters were discussed. In the early 1990s, the United States Food and Drug Administration (FDA) implemented the output display standard.² The FDA's stipulated regulatory upper limits for cardiac applications of 430 mW/cm² for the derated (0.3-dB/cm/MHz) spatial peak and either 1.9 for the mechanical index or 190 mW/cm² (Table 3). The US-induced tissue damage seen in the earlier years of its application shows that lung tissue can be damaged at diagnostic levels. However, through careful and detailed experimental and theoretical studies, it has been shown that the severity of damage is not clinically significant.¹¹³

In the 2000s, the FDA approved therapeutic US for cardiovascular use to treat cardiac arrhythmias and ischemic heart disease with the use of high-intensity focused ultrasound (HIFU) and extracorporeal shock wave therapy (ECWT; Table 4). Although diagnostic US leads to low or negligible increases in tissue temperature, the use of therapeutic US in cardiovascular applications can cause thermal (HIFU) and nonthermal (ECWT) bioeffects.¹¹⁴

The delivery of nanoparticle carriers for drug and gene therapy using microbubbles and US has expanded in recent years. This technology was originally approved for use in echocardiography; the use of a microbubble contrast agents improved the quality of US images due to the difference in acoustic impedance between their gaseous core and the surrounding medium and their nonlinear oscillation in an acoustic field.¹¹⁵ More recently, the use of US in combination with nanoparticles has been shown to enhance the

Table 3. The FDA's Preamendment Levels of Diagnostic US Devices (Modified From O'Brien²)

Application	Derated Intensity Values		
	I _{SPTA} , mW/cm ²	I _{SPPA} , W/cm ²	I _M , W/cm ²
Cardiac	430	190	310

efficacy of drug delivery and reduce side effects of drugs, including treatment of Alzheimer disease, cardiovascular disease, and cancer.¹¹⁶ Although the potential use of high-intensity US for drug delivery can also cause heat, for drug delivery applications, the intensity range of 0.3 to 3 W/cm² is used. The FDA has recommended that an intensity that causes heating of tissues of less than 1°C,¹¹⁶ to avoid heat high intensities, can be applied when the pulse length (pulse cycles/US frequency) and PRF (pulses per second) are reduced.¹¹⁷ Although there are many benefits of using US, US energies higher than cavitation can affect the cell integrity and can thermally and sonochemically induce permanent damage to lipid membranes and cause denaturation of proteins and DNA.¹¹⁸

The first HIFU device was approved by the FDA in October 2004 and, more recently, the use of US with microbubbles for diagnostic applications.¹¹⁶ Therapeutic US for cardiac applications has advanced, especially as an approach to catheter-based ablation of arrhythmias and for treatment of ischemic heart disease.¹¹⁴ The FDA approval process for new medical devices, including therapeutic cardiovascular treatment using US, can be long and tedious. The FDA classifies the devices by risk: (1) low- to moderate-risk devices are typically subjected to what is called premarket notification, also called PMN or 510(k);

and (2) high-risk devices undergo premarket approval, the most stringent type of device application required by the FDA. For the low- to moderate-risk devices, federal law requires new device manufacturers to register with the FDA and notify the agency at least 90 days before they start selling their devices. This premarket notification must prove the device is as safe and effective and substantially equivalent to a similar, legally marketed device. No evidence from clinical studies is needed. For a high-risk or class III device, to gain FDA approval, there must be enough scientific evidence to prove the device is safe and effective for its intended use.¹¹⁹

Studies using pulsed US are clinically important for the identification of an alternative and leadless source of energy for cardiac pacing. Other studies over the years have shown a potential US application for cardiac pacing. Thus, the feasibility and safety of therapeutic US demonstrates its potential for the treatment of cardiovascular diseases.

The exploration of US as an alternative therapy for cardiac pacing in vitro and in vivo up to human studies has shown its potential as a therapeutic technology. This research motivates the development of new therapeutic US applications and advancements. The future of US in the medical field will ultimately depend on collaboration and integration between engineering concepts and cardiovascular studies.

Table 4. Classification of US Waves and Applications in the Medical Field

Classification	US Parameter	FDA-Approved Clinical Applications	Potential Cardiovascular Applications
US intensity	Low-intensity US (<3 W/cm ²)	Therapeutic medicine, imaging medicine, medical diagnosis, drug delivery	Echocardiography
	High-intensity US (<3 W/cm ²)	Surgery, cancer ablation, palliative treatment	Echocardiography with a microbubble contrast agent
US frequency	Low-frequency US (20–200 kHz)	Drug delivery, surgery, cancer ablation, palliative treatment	None
	Medium-frequency US (0.7–3.0 MHz)	Therapeutic medicine, such as bone fracture healing, soft tissue lesion healing, inhibiting inflammatory responses, erectile dysfunction treatment	None
	High-frequency US (1–20 MHz)	Imaging medicine and medical diagnosis, tumor treatment, ECWT for urinary stone treatment	ECWT for ischemic heart disease, peripheral arterial disease, HIFU for atrial fibrillation and SVT ablation, ASD/AVD creation, AVN ablation, functional MR, peripheral vascular disease, ischemic artery disease

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