Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100050893

6. **Project Number and Title of Research Project**: 5 - *Development of the Cardiac Ultrasound Pacemaker*

7. **Start and End Date of Research Project:** 1/1/2010 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Andrew Kohut, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 145,197

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Kohut, Andrew	Principle Investigator	20% Yr1-3	$11,488/yr
Ganguly, Rajarshi	Research Assistant	40% Yr1	$8000
Mehta, Natasha	Research Assistant	30% Yr 3	$5000

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Lewin, Peter	Co-Investigator	10%
Kurnik, Peter	Co-Investigator	5%
Kutalek, Steve	Co-Investigator	5%
Fleischman, Andrew	Research Assistant	30% Yr 1-2

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Custom 2.7 MHz ultrasound probe (Vecchio)	Unique to the institution. This probe will allow stimulation of organs and tissue at specific frequency and energy in tissue culture and animal models.	$2500.00
Custom 1.6 MHz ultrasound probe (Vecchio)	Unique to the institution. This probe will allow stimulation of organs and tissue at specific frequency and energy in tissue culture and animal models.	$2500.00
Focused 2.5 MHz ultrasound probe (Sonic Concepts Inc.)	Unique to the institution. This probe will allow stimulation of organs and tissue at specific frequency and energy in tissue culture and animal models.	$5485.00
Focused 3.5 MHz ultrasound probe (Sonic Concepts Inc.)	Unique to the institution. This probe will allow stimulation of organs and tissue at specific frequency and energy in tissue culture and animal models.	$4910.00
Focused 5.0 MHz ultrasound probe (Sonic Concepts Inc.)	Unique to the institution. This probe will allow stimulation of organs and tissue at specific frequency and energy in tissue culture and animal models.	$4910.00

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____X____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We will continue to move forward with our research with plans of submitting grant proposals to the American Heart Association (AHA) and NIH in 2014-2015.

12. Future of Research Project. What are the future plans for this research project?

We have generated a lot of data in our tissue culture model. We will continue to work with this model and analyze our data further. With the 3 new probes that were purchased, we plan to restart our whole-animal model experiments, in rabbit and sheep models, using the knowledge that we have gained in our porcine and tissue culture models.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes__X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male		1		1
Female				1
Unknown				
Total		**1**		**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic		1		
Non-Hispanic				1
Unknown				1
Total		**1**		**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White				1
Black				
Asian		1		1
Other				
Unknown				
Total		**1**		**2**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No__X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes__X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This research project helped initiate and facilitate research across Drexel University, bringing together clinician-scientists from the School of Medicine and engineers from the School of Biomedical Engineering, Science and Health Systems. With the funding obtained, we now have a number of specialized custom ultrasound probes, providing the opportunity to work with different frequencies, focal lengths and with variable spatial peak time averaged intensities (Ispta) to facilitate further research in a range of animal models, as well as, different tissue models.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

I have initiated discussions for proposed projects with two investigators outside Drexel University. One of the investigators works at Dalhousie University, Halifax, NS, Canada. The second investigator works at Techion University, Israel Institute of Technology, Israel.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

I. Porcine Model Experiments

We initially assembled an experimental cardiac ultrasound pacemaker system and conducted preliminary animal experiments. A diagram of the system appears in Fig. 1. The first system used a 1.6MHz 15mm diameter unfocused transducer and was synchronized to the native cardiac rhythm. Custom gating hardware was designed to detect the QRS complex and deliver the pacing bursts of ultrasound during precise time intervals following each QRS peak (i.e. during ventricular depolarization). The gating hardware was used for two reasons: it allowed us to deliver pacing pulses at a rate somewhat greater than the native rhythm in order to demonstrate the induced pacing effect and, because it prevented the application of pacing pulses during the QRS complex, it lets us avoid inducing tachyarrhythmias via potential early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) . The transducer used in the preliminary experiments is shown in Fig. 2.

First Series of Porcine Experiments

A preliminary series of experiments was performed on 2 porcine models available at our institution utilizing a protocol approved by our University's Institutional Animal Care and Use Committee (IACUC). The following protocol was used for both animals:

Methods

1. Placed 3 ECG electrodes on the shaved chest wall of anesthetized animal
2. Connect ECG electrodes to the stimulating ultrasound and gating hardware
3. Place ultrasound transmission gel and ultrasound probe on chest wall
4. Synchronized ultrasound pulse delivery to native heart rhythm to avoid inducing ventricular arrhythmia. The native porcine heart rate was slightly higher than anticipated, approximately 120-140 bpm.
5. A fixed frequency ultrasound probe of 1.6 MHz was applied using Aquasonic 100® ultrasound transmission gel at approximately the midaxillary line at the level of the 6-8th intercoastal space.
6. Ultrasound bursts were delivered over one minute intervals, using delays of 0.15, 0.2 and 0.3 seconds (corresponding to target heart rates of 400, 300, and 200 BPM respectively) following the peak amplitude of the QRS complex. The pulse durations were set at 2, 4 and 8 milliseconds, while targeting a sound pressure level of 2.5-3.5MPa (megapascal) at the site of stimulation.

a. Ultrasound bursts delivered:

i. 1.6 MHz with pulse delay of 0.15 sec and pulse duration of 2 msec
ii. 1.6 MHz with pulse delay of 0.15 sec and pulse duration of 4 msec
iii. 1.6 MHz with pulse delay of 0.15 sec and pulse duration of 8 msec
iv. 1.6 MHz with pulse delay of 0.20 sec and pulse duration of 2 msec
v. 1.6 MHz with pulse delay of 0.20 sec and pulse duration of 4 msec
vi. 1.6 MHz with pulse delay of 0.20 sec and pulse duration of 8 msec
vii. 1.6 MHz with pulse delay of 0.30 sec and pulse duration of 2 msec
viii. 1.6 MHz with pulse delay of 0.30 sec and pulse duration of 4 msec
ix. 1.6 MHz with pulse delay of 0.30 sec and pulse duration of 8 msec

7. The native heart rhythm was recorded by the 3-lead surface ECG rhythm strip.

8. The ECG rhythm strips were reviewed and analyzed for the presents of ultrasound-initiated beats after each ultrasound pulse parameter change.
9. The heart rhythm was monitored with the electrocardiogram for an additional 5 minutes to document whether any residual electrophysiologic effects from the ultrasound were present.

Results

These preliminary experiments did not result in any clearly identifiable ultrasound-initiated beats as determined by a review of the recordings. The transducer used in these initial tests was likely to have been a contributing factor - its size and shape made it difficult to position accurately on the chest wall and may also have resulted in inconsistencies in ultrasonic coupling. We made several system upgrades in response to this lack of mechanical capture.

Two custom transducers (shown in Figure 3) were constructed for our second series of animal experiments. The new 1.6 MHz transducer is acoustically and electrically identical to the transducer shown in Figure 2 but is mounted in a more compact case which will make it easier to maneuver and position. The second transducer is of the same form factor but operates at 2.5MHz. Figure 4 shows the predicted ultrasonic energy deposition as a function of frequency and skin-to-heart distance. This graph suggests that for an expected anatomical distance of approximately 5 cm, the optimal frequency will be around 0.8MHz. Experiments will be conducted in the frequency range of 0.5MHz to 2.5MHz in order to verify this. We designed and constructed two new ultrasound probes with an improved form factor and purchased additional probes that would allow us to operate at different (potentially more favorable) frequencies. We acquired a new power amplifier (ENI model 3100LA) and signal generator (Agilent model 33220A) that greatly enhanced our ability to broaden the ultrasound exposure matrix and have purchased a new ECG monitor that will provide a clearer signal with a decrease in signal artifact, allowing us to more precisely gate our ultrasound delivery. The rapid native heart rate (up to 140 bpm) encountered in the preliminary experiments may also have interfered with our ability to induce additional beats. We revised the animal protocol to allow for the utilization of different anesthetic agents to provide deeper sedation in an effort to prevent anxiety and associated increased heart rates in our porcine models. This new animal protocol allowed for a broader and more comprehensive protocol per each porcine model, as total experiment time with each animal had been extended to 4 hours, as well as multiple protocols being performed on each animal. This revised animal protocol was approved by the IACUC committee in July 2010.

With our initial prototype and animal protocol we identified several problematic areas/components and tried to directly address these issues.

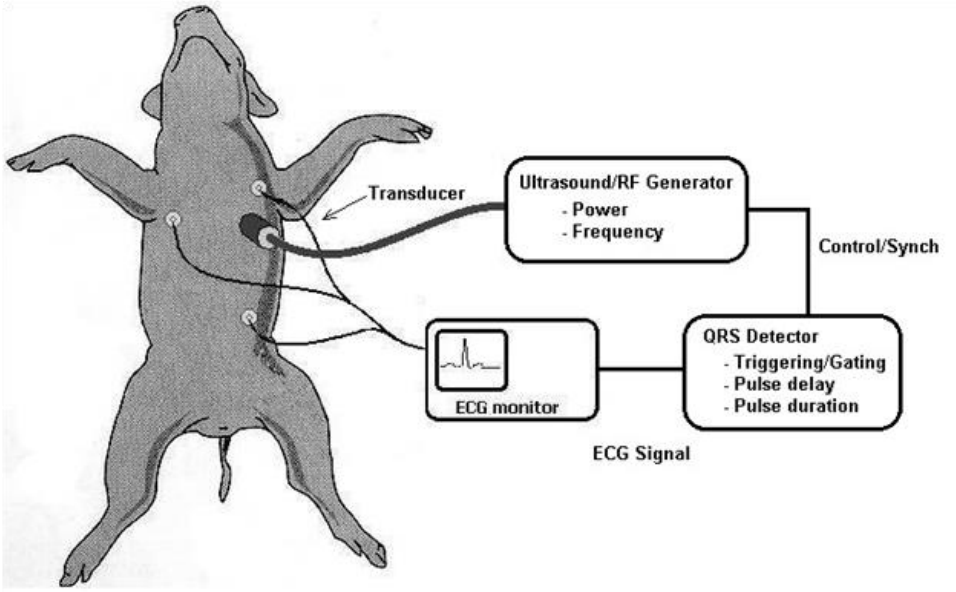

Figure 1. Diagram of Cardiac Ultrasound Pacemaker experimental system.

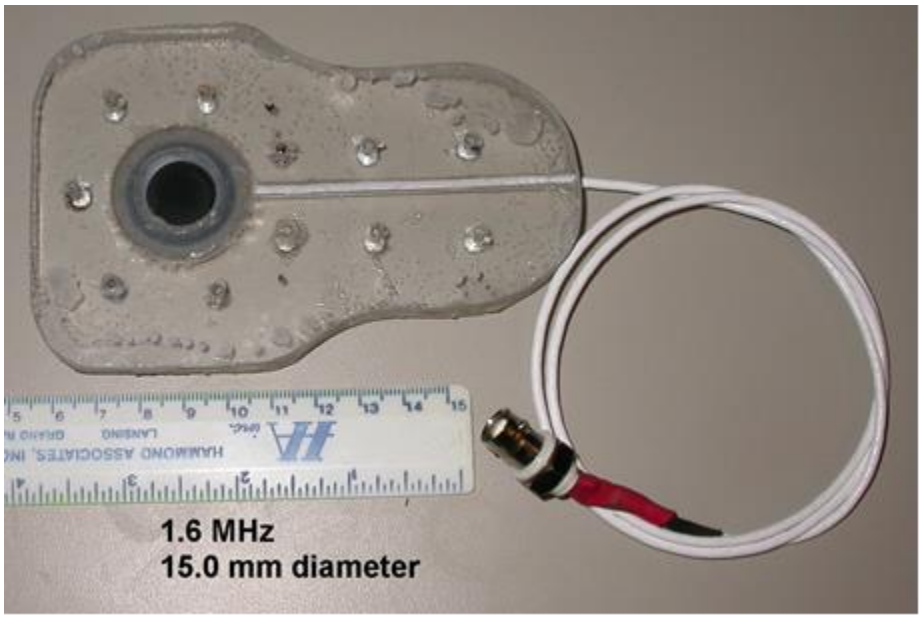

Figure 2. Unfocused, 1.6MHz, 15mm diameter transducer used in the experiments conducted to date.

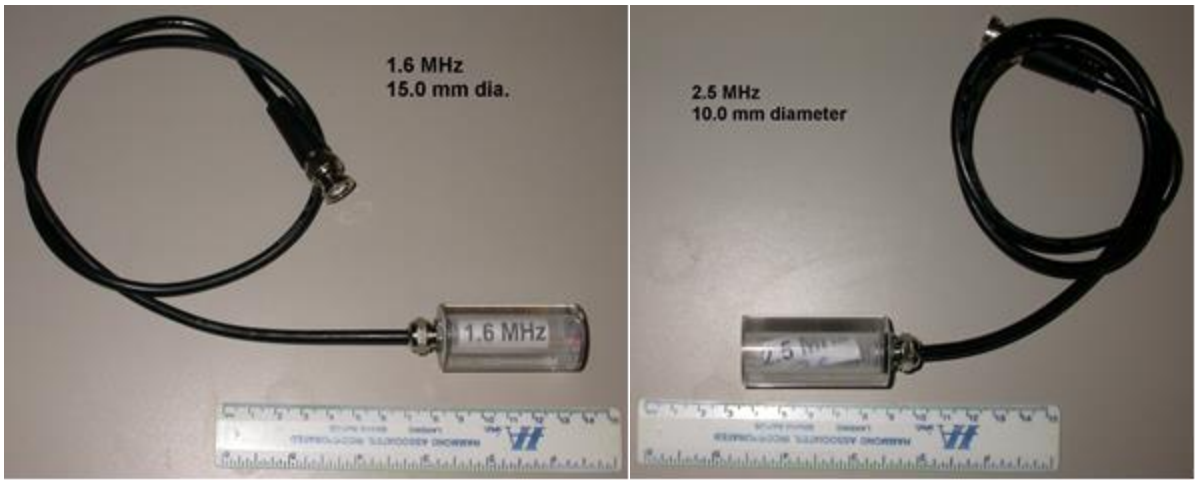

Figure 3. Unfocused, 1.6MHz, 15mm diameter and 2.5MHz, 10mm transducers which will be used in upcoming experiments.

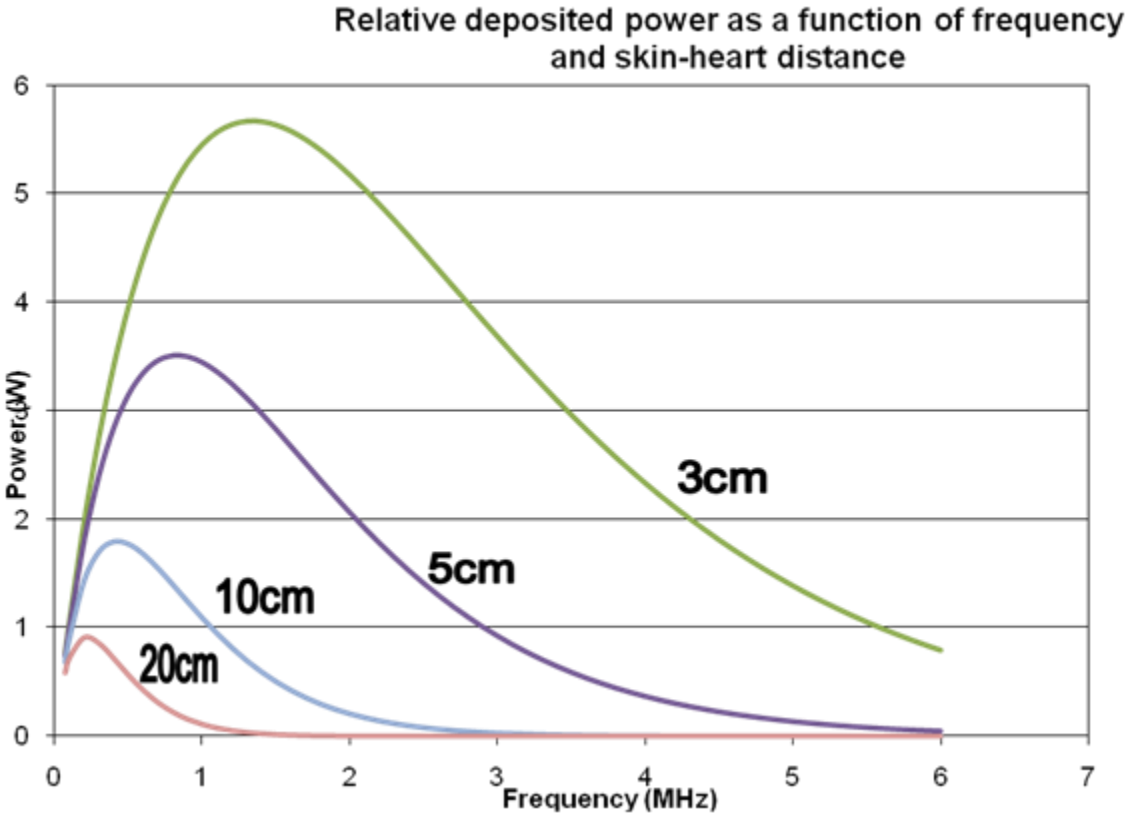

Figure 4. The predicted ultrasonic energy deposition into a 0.5 cm thick heart wall at distances of 3, 5, 10, and 20 cm from the skin surface as a function of frequency. Peak energy deposition for the 5cm curve occurs at a frequency of approximately 0.8MHz.

Second Series of Porcine Experiments

Two modified animal protocols were approved by the IACUC committee in July 2010 and September 2010. The protocols are described in more detail below.

Methods

I. A series of experiments was performed on 4 porcine models utilizing a protocol approved by our University's Institutional Animal Care and Use Committee (IACUC). The following protocol was used for these animals:

1. Placed 3 ECG electrodes on the shaved chest wall of anesthetized animal
2. Connected ECG electrodes to ECG monitor and ECG monitor to QRS detection, gating, and stimulating ultrasound hardware
3. Placed ultrasound transmission gel and ultrasound probe on chest wall
4. Synchronized ultrasound pulse delivery to native heart rhythm to avoid inducing ventricular arrhythmia. The native porcine heart rate was slightly higher than anticipated in some instances, approximately 120-140 bpm.
5. A fixed frequency ultrasound probe of either 1.6 MHz or 2.5 MHz was applied using Aquasonic 100® ultrasound transmission gel at 4 different locations on the chestwall: (1) midclavicular line at the level of the 6-8th intercoastal space, (2) midaxillary line at the level of the 6-8th intercoastal space and (3) midclavicular line from subcoastal angulation towards the ipsilateral should. These sites of stimulation were selected using 2D echocardiography.

a. Ultrasound pulses were delivered for one minute bursts, with pulse delays of 0, 0.05, 0.10, 0.15, 0.2 and 0.3 seconds, following the peak amplitude of the QRS complex. The pulse durations were set at 2, 4, 8, 10 and 14 milliseconds, while targeting a sound pressure level of 2.5-3.5MPa (megapascal) at the sites of stimulation.

b. Two animals were exposed to ultrasound pulses delivered:

i. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 2 msec
ii. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 3 msec
iii. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 4 msec
iv. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 10 msec
v. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 12 msec
vi. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 14 msec
vii. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 2 msec
viii. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 4 msec
ix. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 10 msec
x. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 12 msec
xi. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 14 msec

c. Two animals were exposed to ultrasound pulses delivered:

i. 2.5 MHz with pulse delay of 0 sec and pulse duration of 2 msec
ii. 2.5 MHz with pulse delay of 0 sec and pulse duration of 4 msec
iii. 2.5 MHz with pulse delay of 0 sec and pulse duration of 8 msec
iv. 2.5 MHz with pulse delay of 0 sec and pulse duration of 10 msec
v. 2.5 MHz with pulse delay of 0 sec and pulse duration of 14 msec
vi. 2.5 MHz with pulse delay of 0.20 sec and pulse duration of 2 msec
vii. 2.5 MHz with pulse delay of 0.20 sec and pulse duration of 4 msec
viii. 2.5 MHz with pulse delay of 0.20 sec and pulse duration of 8 msec
ix. 2.5 MHz with pulse delay of 0.20 sec and pulse duration of 10 msec

x. 2.5 MHz with pulse delay of 0.20 sec and pulse duration of 14 msec
xi. 2.5 MHz with pulse delay of 0.30 sec and pulse duration of 2 msec
xii. 2.5 MHz with pulse delay of 0.30 sec and pulse duration of 4 msec
xiii. 2.5 MHz with pulse delay of 0.30 sec and pulse duration of 8 msec
xiv. 2.5 MHz with pulse delay of 0.30 sec and pulse duration of 10 msec
xv. 2.5 MHz with pulse delay of 0.30 sec and pulse duration of 14 msec
6. The native heart rhythm was recorded using 3-lead surface ECG rhythm strip.
7. The ECG rhythm strips were reviewed and analyzed for the presents of ultrasound-initiated beats after each ultrasound pulse parameter change.
8. The heart rhythm was monitored with the electrocardiogram for an additional 5 minutes to document whether any residual electrophysiologic effects from the ultrasound are present.

II. A second series of experiments was performed on 2 porcine models utilizing a protocol approved by our University's Institutional Animal Care and Use Committee (IACUC). The following protocol was used for these animals:
1. Each animal was anesthetized, intubated and placed on a mechanical respirator
2. Placed 3 ECG electrodes on the shaved chest wall of anesthetized animal
3. Connected ECG electrodes to ECG monitor and ECG monitor to QRS detection, gating, and stimulating ultrasound hardware
4. Sternotomy performed using standard sterile surgical technique. The pericardial sack was resected and a 3-4 cm diameter of the epicardial surface of the left ventricle was exposed.
5. A fluid-filled latex sack, as a functional spacer, was placed in direct contact with the surface of the left ventricle. A fixed frequency ultrasound probe of either 1.6 MHz or 2.5 MHz was applied to the latex sack
6. Synchronized ultrasound pulse delivery to native heart rhythm to avoid inducing ventricular arrhythmia. The native porcine heart rate was slightly higher than anticipated in some instances, approximately 120-140 bpm.
a. Ultrasound pulses were delivered for one minute bursts, with pulse delays of 0.2 and 0.3 seconds, following the peak amplitude of the QRS complex. The pulse durations were set at 2, 4, 10, 12 and 14 milliseconds, while targeting a sound pressure level of 2.5-3.5MPa (megapascal) at the sites of stimulation.
b. Each animal was exposed to ultrasound pulses delivered:
i. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 2 msec
ii. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 4 msec
iii. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 10 msec
iv. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 12 msec
v. 1.6 MHz with pulse delay of 0.2 sec and pulse duration of 14 msec
vi. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 2 msec
vii. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 4 msec
viii. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 10 msec
ix. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 12 msec
x. 1.6 MHz with pulse delay of 0.3 sec and pulse duration of 14 msec

Results
Unfortunately, the results of these experiments remained inconclusive. No clearly identifiable ultrasound-initiated beats were seen in the ECG recordings. In an effort to improve our system, we mathematically modeled the generated ultrasound beam intensities and accounted for the energy losses incurred as the beam passes through the porcine chest wall and cardiac tissue layers. Our data from this model suggested that lower frequency ultrasound may be more likely to deliver the required radiation force to the epicardial and endocardial layers of the heart.

At this point in time, we purchased a new lower frequency (0.5MHz), focused ultrasound probe. This is a high-efficiency; broad bandwidth transducer designed for high-intensity focused ultrasound experiments. This new probe would allow us to investigate the utility of lower frequency ultrasound excitations and, as a result of focusing, allow us to overcome the effects of attenuation to apply higher ultrasound intensities at the epicardial surface.

Third Series of Porcine Experiments
The animal protocol was approved by the Drexel University College of Medicine's Institutional Animal Care and Use Committee (IACUC) on June 20, 2011. The protocol is described in more detail below.

Methods
I). Another series of experiments was performed on 4 porcine models utilizing a protocol approved by our University's IACUC. The following protocol was used for these animals:
1. Placed 3 ECG electrodes on the shaved chest wall of anesthetized animal
2. Connected ECG electrodes to ECG monitor and ECG monitor to QRS detection, gating, and stimulating ultrasound hardware
3. Placed ultrasound transmission gel and ultrasound probe on chest wall
4. Synchronized ultrasound pulse delivery to native heart rhythm to avoid inducing ventricular arrhythmia. The native porcine heart rate was slightly higher than anticipated in some instances, approximately 120-140 bpm.
5. A fixed frequency ultrasound probe of 2.5Mz and a second ultrasound probe of 0.5 MHz, with and without a 3rd harmonic matching network (1.625 MHz), were applied using Aquasonic 100® ultrasound transmission gel at 4 different locations on the chestwall: (1) midclavicular line at the level of the 6-8th intercoastal space, (2) midaxillary line at the level of the 6-8th intercoastal space, (3) midclavicular line from subcoastal angulation towards the ipsilateral should and (4) midaxillary line from subcoastal angulation towards the ipsilateral should . These sites of stimulation were selected using 2D echocardiography.
a. Ultrasound pulses were delivered for one minute bursts, with pulse delays of 0, 0.2, 0.4, 0.5 and 0.6 seconds, following the peak amplitude of the QRS complex. The voltage was set to 100mV, 300mV, 500mV, 700mV and 1V (these voltages represent peak-peak values). The pulse durations were maintained at 10 milliseconds, while targeting a sound pressure level of 2.5-3.5MPa (megapascal) at the sites of stimulation.
b. Two animals were exposed to ultrasound pulses delivered:
i. 2.5 MHz with pulse delay of 0.2 sec and 500mV
ii. 2.5 MHz with pulse delay of 0.4 sec and 500mV
iii. 2.5 MHz with pulse delay of 0.5 sec and 500mV

iv. 2.5 MHz with pulse delay of 0.6 sec and 500mV
v. 2.5 MHz with pulse delay of 0.2 sec and 1V
vi. 2.5 MHz with pulse delay of 0.4 sec and 1V
vii. 2.5 MHz with pulse delay of 0.5 sec and 1V
viii. 2.5 MHz with pulse delay of 0.6 sec and 1V

c. Two animals were exposed to ultrasound pulses delivered:

i. 0.5 MHz with pulse delay of 0.2 sec and 100mV
ii. 1.625 MHz with pulse delay of 0.2 sec and 100mV
iii. 0.5 MHz with pulse delay of 0.2 sec and 300mV
iv. 1.625 MHz with pulse delay of 0.2 sec and 300mV
v. 0.5 MHz with pulse delay of 0.2 sec and 500mV
vi. 1.625 MHz with pulse delay of 0.2 sec and 500mV
vii. 0.5 MHz with pulse delay of 0.2 sec and 700mV
viii. 1.625 MHz with pulse delay of 0.2 sec and 700mV
ix. 0.5 MHz with pulse delay of 0.2 sec and 1V
x. 1.625 MHz with pulse delay of 0.2 sec and 1V
xi. 0.5 MHz with pulse delay of 0.4 sec and 100mV
xii. 1.625 MHz with pulse delay of 0.4 sec and 100mV
xiii. 0.5 MHz with pulse delay of 0.4 sec and 300mV
xiv. 1.625 MHz with pulse delay of 0.4 sec and 300mV
xv. 0.5 MHz with pulse delay of 0.4 sec and 500mV
xvi. 1.625 MHz with pulse delay of 0.4 sec and 500mV
xvii. 0.5 MHz with pulse delay of 0.4 sec and 700mV
xviii. 1.625 MHz with pulse delay of 0.4 sec and 700mV
xix. 0.5 MHz with pulse delay of 0.4 sec and 1V
xx. 1.625 MHz with pulse delay of 0.4 sec and 1V
xxi. 0.5 MHz with pulse delay of 0.5 sec and 100mV
xxii. 1.625 MHz with pulse delay of 0.5 sec and 100mV
xxiii. 0.5 MHz with pulse delay of 0.5 sec and 300mV
xxiv. 1.625 MHz with pulse delay of 0.5 sec and 300mV
xxv. 0.5 MHz with pulse delay of 0.5 sec and 500mV
xxvi. 1.625 MHz with pulse delay of 0.5 sec and 500mV
xxvii. 0.5 MHz with pulse delay of 0.5 sec and 700mV
xxviii. 1.625 MHz with pulse delay of 0.5 sec and 700mV
xxix. 0.5 MHz with pulse delay of 0.5 sec and 1V
xxx. 1.625 MHz with pulse delay of 0.5 sec and 1V
xxxi. 0.5 MHz with pulse delay of 0.6 sec and 100mV
xxxii. 1.625 MHz with pulse delay of 0.6 sec and 100mV
xxxiii. 0.5 MHz with pulse delay of 0.6 sec and 300mV
xxxiv. 1.625 MHz with pulse delay of 0.6 sec and 300mV
xxxv. 0.5 MHz with pulse delay of 0.6 sec and 500mV
xxxvi. 1.625 MHz with pulse delay of 0.6 sec and 500mV
xxxvii. 0.5 MHz with pulse delay of 0.6 sec and 700mV
xxxviii. 1.625 MHz with pulse delay of 0.6 sec and 700mV
xxxix. 0.5 MHz with pulse delay of 0.6 sec and 1V
xl. 1.625 MHz with pulse delay of 0.6 sec and 1V

6. The native heart rhythm was recorded using 3-lead surface ECG rhythm strip.
7. The ECG rhythm strips were reviewed and analyzed for the presents of ultrasound-initiated beats after each ultrasound pulse parameter change.
8. The heart rhythm was monitored with the electrocardiogram for an additional 5 minutes to document whether any residual electrophysiological effects from the ultrasound are present.

III. An additional series of experiments was performed on 2 porcine models utilizing a protocol approved by our University's IACUC. The following protocol was used for these animals:
1. Each animal was anesthetized, intubated and placed on a mechanical respirator
2. Placed 3 ECG electrodes on the shaved chest wall of anesthetized animal
3. Connected ECG electrodes to ECG monitor and ECG monitor to QRS detection, gating, and stimulating ultrasound hardware
4. Sternotomy performed using standard sterile surgical technique. The pericardial sack was resected and a 3-4 cm diameter of the epicardial surface of the left ventricle was exposed.
5. A fluid-filled latex sack, as a functional spacer, was placed in direct contact with the surface of the left ventricle. A fixed frequency ultrasound probe of 0.5 MHz, with and without a 3rd harmonic matching network (1.625 MHz), was applied to the latex sack
6. Synchronized ultrasound pulse delivery to native heart rhythm to avoid inducing ventricular arrhythmia. The native porcine heart rate was slightly higher than anticipated in some instances, approximately 120-140 bpm.

a. Ultrasound pulses were delivered for one minute bursts, with pulse delays of 0.1, 0.2, 0.3, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 and 1.0 seconds, following the peak amplitude of the QRS complex. The voltage was set to 500mV and 1V (these voltages represent peak-peak values). The pulse durations were maintained at 10 milliseconds, while targeting a sound pressure level of 2.5-3.5MPa (megapascal) at the sites of stimulation. This series of ultrasound bursts was repeated three times in each animal.

Results

Unfortunately, the results of our porcine model experiments remained inconclusive. No clearly identifiable ultrasound-initiated beats were seen in the ECG recordings. In an effort to improve our system, we again mathematically modeled the generated ultrasound beam intensities and accounted for the energy losses incurred as the beam passes through the porcine chest wall, adipose, connective and cardiac tissue layers. Our revised model includes additional information derived from our completed experiments. Instead of using a porcine model, we are planning to use an ovine model and a rabbit model, as we think that sheep and rabbit will be more appropriate models for mechanoelectrical stimulation of cardiac tissue based on recent findings in the literature and discussions with expert consultants.

II. Tissue Culture Model Experiments

Rational for changing our experimental design and working in a tissue culture model:
With our prior lack of success in the porcine model, we decided to reassess our initial assumptions regarding ultrasound and its effects on cells by initiating a series of tissue culture experiments using rat ventricular cardiomyocytes. We used the same custom 2.5 MHz high-intensity focused ultrasound (HIFU) probe that was used in the porcine experiments previously described. In an effort to deliberately attenuate the energy that reached the cardiomyocytes in tissue culture we used a holder for the probe that is setback/spacer from the tissue culture plate, as well as, lower energy parameters. With the information obtained from these experiments in tissue models, we plan to modify our ultrasound parameters in our anticipated rabbit and sheep model experiments planned for 2014-2015.

Methods

Harvesting and Cell Preparation

Neonatal ventricular rat cardiomyocytes were harvested from excised hearts of 1 to 3 day old euthanized Sprague-Dawley rat pups using a cell isolation kit (Cellutron Life Technology, Baltimore, MD). Briefly, cells were isolated from the cardiac tissue of both ventricles. The cardiomyocytes were pre-plated for 1 to 2 h with SureCoat (Cellutron) to purify the cardiomyocytes population from interspersed fibroblasts. The cardiomyocytes were cultured at a density of 7,000 cells/cm2 in high serum medium (10% fetal bovine serum) (Cellutron NS medium) in 60 mm cell culture dishes with gel substrates and placed in an incubator for 24 h at 5% CO2 and 37ºC. The medium was changed to low serum (2% fetal bovine serum) (Cellutron NW medium) and maintained for another 24 h in the incubator. This waiting period proved sufficient to allow the cardiomyocytes to attach and spread completely after the isolation procedure. For long-term maintenance of cardiomyocytes in culture, the media were replaced with fresh low serum media every 2 days. Bromodeoxyuridine (BrdU) was added to these media at a concentration of 200 µM to prevent fibroblast proliferation.

Prior to experimentation, the medium was removed and the cardiomyocytes were washed with Tyrode's solution. The cells were stained for 10 minutes with annine-6plus (Sensitive Dyes GBR, Munich, Germany) dye with strong membrane binding affinity. This dye became concentrated at the nuclear membrane of the cardiomyocytes, and helped to visualize and characterize the beating cells. After staining, the culture was washed three times with Tyrode's solution. Finally, the cardiomyocytes were equilibrated in Tyrode's solution for 1 to 2 hrs inside an incubator. This time period proved sufficient to restore the automaticity of the cardiomyocytes culture without compromising the cardiomyocytes in the culture due to limited nutrition. The cardiomyocytes were kept in the incubator at 37°C until just prior to the experiment.

Mechanical Deformation Protocol

A setback/spacer was formed using a modified syringe as a mold with 3% agarose and water. This spacer allowed the ultrasound to be delivered from its appropriate focal distance (2.0 cm) to the well plate with minimal attenuation or reflection (ultrasound does not conduct well through air). A custom 2.5 MHz high-intensity focused ultrasound (HIFU) probe (Vecchio)

was coupled to the spacer with a thin layer of liquid water and grossly aimed at the center of the well plate at a distance of 2.0cm. The transducer was later focused on various confluent areas within the well plate using the microscope stage adjustments. The well plates contained high volumes of Tyrode's solution to act as a conducting medium for the ultrasound without risk of a large acoustic impedance mismatch.

The transducer was controlled via a function generator (Agilent 33220A). Sinusoidal waveforms at a frequency of 2.5 MHz were generated by the function generator and amplified +51 dB before reaching the transducer. The function generator was set to deliver acoustic energy at variable spatial peak time averaged intensities (Ispta) in W/cm2 at pulse durations of 1, 2, 3 or 5ms, with a burst period (pulse repetition period) of 300ms. This pulse rate suggests delivery of 200 ultrasound pulses per minute. Unfortunately, we were not able to initiate ultrasound pulses at a specific phase of the action potential of the cardiomyocytes, as has been achieved in whole heart studies using electrocardiogram leads and a relay. Instead, pulses were initiated by a timer and delivered at 300ms fixed intervals.

Data Recording and Analysis

A Nikon Diaphot 300 inverted phase contrast microscope was connected to a high speed Dalsa CA-D1-0128T camera with NI-1422 frame-grabber. Timing and image capture from the CCD were controlled via custom NI LabviewVI. This setup recorded 12 bit, 128x127 pixel arrays at a frame rate of 155 frames per second for 8 seconds. Chroma 86000 series filters were used with a mercury lamp to excite the annine-6plus dye at about 490 nm and emitted light was directed through a 617/70 filter. For each trial, frames were captured for 8 seconds without ultrasound exposure to establish a control. Immediately following this control, the same sample was exposed to ultrasound while capturing an additional 8 seconds of frames. These frames were further analyzed using software from ImageJ (NIH).
The contraction rate of the cardiomyocytes was measured based upon the translocation of the nuclear membrane captured by this high-speed camera. A pattern detection algorithm (Image J and a custom Matlab and C software package) translated captured images into beat frequency, measured as beats per minute (BPM).

An average of the duration of the cardiac cycle from all recorded beats was calculated for both the control and ultrasound exposure groups. Statistical comparisons were made between controls prior to ultrasound exposure and after exposure for each trial using two sample t-tests with unequal variance. Results from individual trials were pooled for each set of conditions and analyzed with a Wilcoxon Signed Rank Test. P values < 0.05 were considered statistically significant.

Results

The cardiomyocyte contraction rate, defined as beat frequency and measured as beats per minute (BPM), was recorded for 11 different ultrasound parameters, which included variable spatial peak time, averaged intensities (Ispta) and pulse duration (1, 2, 3 and 5ms). Individual trials were first analyzed independently, and then the results from all trials using the same parameters were pooled. Finally, all conditions with a single matching parameter were grouped to isolate that parameter and characterize its contribution to possible changes in cardiomyocyte beat frequency.

Individual trials

Individual trials using a single set of ultrasound parameters were repeated between 3 and 13 times and the resulting beat rate of the cardiomyocytes was compared to controls from that same monolayer of cardiomyocytes just prior to ultrasound exposure. Table 1 summarizes the results of all 99 trials and the influence of ultrasound exposure on beat frequency. Of the 99 trials, the beat frequency increased with respect to controls in 57 trials; however, the beat rate decreased in 42 trials. Only 19 trials showed a significant change ($P < 0.05$) from control; 11 trials with a significant increase and 8 with a significant decrease. Of the 11 trials showing a significant increase in beat frequency, 10 of these trials employed ultrasound intensities of 0.2 W/cm2 or less. Also, 4 of the 8 trials showing a statistically significant decrease in beat frequency utilized an input voltage of 0.2W/cm2 or greater. A ratio of the beat frequency during ultrasound exposure to the control prior to exposure was determined for each trial. The trials showing an increase in beat frequency had a mean ratio of 1.246 (range of 1.059 to 1.504), suggesting a mean increase in beat frequency of 24.6%. This is disparate from the trials showing a decrease in beat frequency, which demonstrated a mean ratio of 0.908 (range of 0.792 to 0.954), representing a mean decline of only 9.20%. Only 13 of 42 associated trials showed a greater than 10% decrease in beat frequency during exposure to ultrasound, whereas 26 of 57 trials showed a greater than 15% increase in beat frequency.

Table 1. Summary of results from 99 trials using 11 ultrasound parameters

I_{spta} (W/cm^2)	Pulse Duration	# of Trials	Increase in BPM	Significant Increase*	Ratio	Decrease in BPM	Significant Decrease*	Ratio
0.02	1 ms	13	10 (76.9%)	3	1.387	3 (23.1%)	0	0.922
0.04	1 ms	12	8 (66.7%)	1	1.184	4 (33.3%)	0	0.873
0.05	3 ms	10	3 (30.0%)	1	1.145	7 (70.0%)	1	0.954
0.07	1 ms	9	3 (33.3%)	0	1.247	6 (66.7%)	2	0.939
0.08	2 ms	3	3 (100%)	1	1.359	0 (0.00%)	0	~
0.09	5 ms	12	9 (75.0%)	2	1.169	3 (25.0%)	1	0.878
0.12	3 ms	12	7 (58.3%)	1	1.348	5 (41.7%)	0	0.928
0.20	5 ms	8	5 (62.5%)	1	1.252	3 (37.5%)	1	0.794
0.21	3 ms	3	1 (33.3%)	0	1.162	2 (66.7%)	0	0.792
0.35	5 ms	13	5 (38.5%)	0	1.059	8 (61.5%)	3	0.905
0.87	2 ms	4	3 (75.0%)	1	1.504	1 (25.0%)	0	0.942
		99	57 (57.6%)	11	1.246	42 (42.4%)	8	0.908

*Statistically significant ($p < 0.05$) - 2 sample t-test with unequal variance

Pooled Trials

After analyzing all 99 trials independently, repeated trials using the same ultrasound parameters were pooled for analysis. Table 2 summarizes the results of all 11 conditions and the average beat frequency from all pooled trials before and after exposure to ultrasound. The beat frequency increased relative to the control in 8 of the 11 conditions. Of these conditions, the ratio of beat rate after ultrasound exposure relative to the control ranged from 1.007 to 1.292, with 3 conditions showing an increase over 20%. However, only a single condition (0.02 W/cm2, 1 ms pulse duration) was determined to be statistically significant ($p < 0.05$) in producing an increase in beat frequency. Similar to the results from individual trials, all 3 conditions with a decline in beat rate employed acoustic intensities greater than 0.2 W/cm2,

however none reached statistical significance. The remainder of ultrasound voltages showed an increase in beat frequency, without achieving statistical significance.

Table 2. Summary of results after combining all trials using the same conditions

I_{spta} (W/cm^2)	Pulse Duration	# of Trials	Control BPM	Ultrasound BPM	Ratio	P Value
0.02	1 ms	13	72.28	87.81	1.215	0.004*
0.04	1 ms	12	44.79	45.70	1.020	0.319
0.05	3 ms	10	64.21	64.66	1.007	0.508
0.07	1 ms	9	67.58	67.53	0.999	0.767
0.08	2 ms	3	60.73	78.47	1.292	0.054
0.09	5 ms	12	45.46	47.95	1.055	0.120
0.12	3 ms	12	53.67	59.03	1.100	0.079
0.20	5 ms	8	49.03	49.69	1.013	0.337
0.21	3 ms	3	51.85	45.03	0.869	0.593
0.35	5 ms	13	92.37	89.10	0.965	0.196
0.87	2 ms	4	37.03	46.54	1.257	0.137

*Statistical significance ($P < 0.05$- Wilcoxon Signed Rank Test)

Discussion

In this study, we attempted to influence the beat frequency of cultured rat ventricular cardiomyocytes using a source of pulsed high-intensity focused ultrasound (HIFU). Neonatal rat cardiomyocytes retain an inherent rhythmicity in culture. Some sources suggest an in-vitro rate of contraction of 43 ± 21 bpm. Although ultrasound pulses were delivered at a rate of 200 per minute, they were not synchronized with the resting cardiomyocyte beat frequency. Thus, we did not expect to capture and pace the cardiomyocytes up to a frequency of 200 beats per minute.

Of the 99 individual trials, 19 trials produced a change in beat frequency that was statistically significant. In 13 of these trials, ultrasound produced an increase in beat frequency and in 8 trials ultrasound produced a decrease in beat frequency. In the 57 trials that showed an increase in beat frequency, there was a mean increase of contraction frequency of 24.6%. In the 42 trials that demonstrated a decrease in beat frequency, there was a mean decrease of 9.2%. The 3 ultrasound parameters that caused a decrease in beat frequency employed acoustic intensities greater than 0.2 W/cm2. Specific ultrasound parameters (intensity and pulse duration) caused different changes in beat frequency, some potentiating the beat frequency, while other parameters inhibited the beat frequency. The lowest intensity of ultrasound exposure, 0.02 W/cm2 and 1 ms pulse duration, was most effective at increasing the beat frequency.

Our data suggests that low (2.5 MHz) frequency ultrasound has a modulating effect on the rate of cardiomyocyte contraction. Rate-response varied depending on the amount of acoustic power and pulse duration. The lowest level of acoustic power and shortest pulse duration tested proved most effective at increasing the responsiveness and beat frequency of cardiomyocytes.

III. Additional Tissue Culture Experiments Focused on Analyzing Cardiomyocyte Depolarization Rates

Methods

Neonatal ventricular rat cardiomyocytes were harvested from excised hearts of 1 to 3 day old euthanized Sprague-Dawley rat pups using a cell isolation kit (Cellutron Life Technology, Baltimore, MD). Briefly, cells were isolated from the cardiac tissue of both ventricles. The cardiomyocytes were pre-plated for 1 to 2 h with SureCoat (Cellutron) to purify the cardiomyocytes population from interspersed fibroblasts. The cardiomyocytes were cultured at a density of 7,000 cells/cm2 in high serum medium (10% fetal bovine serum) (Cellutron NS medium) and plated directly on microelectrode arrays (MEAs) embedded with titanium nitrite electrodes for EKG readings. The custom 2.5 MHz high-intensity focused ultrasound (HIFU) probe, used in the previously described experiments, was directed at the cardiomyocytes in a tissue culture model. A piezoelectric metal plate 3cm in diameter was on the exposure end. The transducer was placed in a setback/spacer, which was a water-filled 50ml polypropylene centrifuge tube with a polyethylene pipette attached to the conical end directed at a 45-degree angle over the MEA. There was a continuous fluid column from the surface of the ultrasound probe through the setback/spacer and to the plate filled with medium and monolayer of cardiomyocytes. The pipette tip was inserted into the medium approximately 2-3mm above the monolayer of cardiomyocytes. A function generator, with an amplified signal +50 dB, delivered acoustic energy at variable settings of 0.1, 0.3, 0.5 and 1.0 Vpp, pulse durations of 2, 5 and 10ms, and burst periods of 100, 250 and 300ms. At least five trials were conducted at each setting (180 total trials) with 30s of continuous ultrasound exposure followed by an off interval of 30-60 seconds. Temperature of the medium was monitored throughout the trials. The MEA electrodes were connected to a custom software package that produced recording of continuous electrical activity (action potentials) from pre-specified regions on the surface of the MEA plate. The continuous recordings of cardiomyocyte electrical activity was recorded and subsequently analyzed.

Results

The interval durations (ID) between action potentials were measured throughout the recording period and subsequently analyzed. Prior to ultrasound delivery, the IDs were highly irregular, ID range = 0.3-2.7 s. As ultrasound was delivered in an asynchronous manner, using 0.1 and 0.3 Vpp and PD = 2 and 5 ms, there was suppression/inhibition of cellular depolarization for the first 5-10 s. Then 10-15 s after the start of ultrasound delivery, the depolarization rate increased and demonstrated less interval duration variability (ID=0.88-1.03 s, P value<0.05), even after the ultrasound exposure. The data analysis of this series of experiments is ongoing at this point in time.

IV. Conclusions

Unfortunately, the results of our animal experiments were inconclusive, as there was no clearly identifiable ultrasound-initiated beats seen in the ECG recordings. However, our experiments in tissue culture model produced exciting results; supporting the idea that low (2.5 MHz) frequency ultrasound has a modulating effect on the rate of cardiomyocyte

contraction. The rate-response of cardiomyocyte contractility varied depending on the amount of acoustic power and pulse duration. The lowest level of acoustic power and shortest pulse duration tested proved most effective at increasing the responsiveness and beat frequency of cardiomyocytes in a tissue culture model. In addition, our second series of tissue culture experiments using MEAs generated data suggesting that ultrasound can inhibit and modify the frequency of spontaneous electrical depolarizations of neonatal ventricular cardiomyocytes. Ultrasound-induced radiation force stimulating the cardiomyocytes via stretch and compression-mediated mechanosensitive pathways is a plausible mechanism of action. Ultrasound may prove to be a practical, noninvasive tool for applying mechanical force to myocardial tissue for clinical and research applications.

We recently purchased 3 new high-intensity focused ultrasound (HIFU) probes based on our research findings in porcine and tissue culture models. One of these probes is pictured in Figure 5. We plan to initiate a series of experiments in sheep and rabbits later this year using these new probes.

Figure 5. A high-efficiency, broad bandwidth transducer intended for high-intensity focused ultrasound (HIFU) experiments.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed

acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

We have written one manuscript, entitled *Ultrasound-Induced Manipulation of Cardiac Rhythm in Neonatal Rat Ventricular Cardiomyocytes*. This manuscript is currently being revised and with plans for submission in the next several months.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Our research findings in the tissue culture model are novel and hold potential for several future clinical applications. We have been able to use ultrasound to stimulate, inhibit and modify the frequency of spontaneous electrical depolarizations of neonatal ventricular cardiomyocytes in a tissue culture model. Our observations could be due to conditioning via stretch and compression-mediated mechanosensitive pathways, by modifying intracellular

calcium handling or altering cell signaling.

We are currently working on characterizing these ultrasound-induced effects and outlining different potential cellular mechanisms which could be mediating these cellular responses. Our results suggest that focused ultrasound might hold therapeutic potential in modulating electrical activation and propagation in cardiac tissue.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No __X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No_____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No_____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Kohut, Andrew Roman	POSITION TITLE Assistant Professor of Medicine
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Pennsylvania	B.A.	05/94	Biology
Boston University	M.P.H.	06/98	Epidemiology
MCP Hahnemann School of Medicine	M.D.	06/02	

A. Positions and Honors

Positions:

2002-2005: Resident, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA.

2005-2006: Chief Resident, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA.

2006-2009: Fellow, Division of Cardiology, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA.

2009-present: Assistant Professor of Medicine, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA.

2012-present: Medical Director of Nuclear Cardiology, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA.

Honors:

1990-1994	CIGNA Citizen's Scholarship
1991	Grass Instruments Scholarship
1990, 1993, 1994	Dean's Honor List
1994	Cum Laude, with Honor's Distinction in Biology
2003	Best Regional Abstract, Journal of Nuclear Cardiology
2006	Alpha Omega Alpha Honor Medical Society
2007	Golden Apple Award Nominee, Class of 2007
2010	Golden Apple Award Nominee, from the Class of 2010
2010	Golden Apple Award Nominee, from the Class of 2011
2011	Golden Apple Award Nominee, from the Class of 2011
2011	Dean’s Special Award for Excellence in Clinical Teaching

B. Peer-reviewed Publications

Full Length Papers:

1. Perfusion and Wall Motion Abnormalities in a Patient with Diabetic Ketoacidosis. A. Kohut and D. Jain. J Nucl Cardiol, 2009, Volume 16, Number 2: 316-320.
2. Percutaneous extraction of ePTFE-coated ICD leads; a single center comparative experience. A. Kohut, J. Grammes, C. Schulze, M. Al-Bataineh, G. Yesenosky, J. Harrow, and S. Kutalek. Pacing and Clinical Electrophysiology. 2013; 36(4):444-450.

Abstracts:

1. Preventing apoptosis should constitute the basis of management of atherosclerosis: pancaspase inhibitor simulate the effects of diet withdrawal and statin therapy. D. Hartung, A. Petrov, S. Edwards, F. Kolodgie, N. Narula, A. Kohut, N. Haider, R. Virmani, J. Narula. J Nucl Cardiol, 2003, Volume 10, Number 1, S22, Suppl.
2. Abrogation of apoptosis in atherosclerotic plaques: feasibility of noninvasive detection by radionuclide imaging with annexin-V. D. Hartung, A. Petrov, F. Kolodgie, N. Narula, A. Kohut, N. Haider, H. Gold, R. Virmani, J. Narula. JACC, 2003, Volume 41, Number 6 Suppl. A, 445A (835-4).
3. The ePTFE-coated ICD leads are easier to remove than standard ICD leads up to 50 months implant duration. A. Kohut, J. Rafeek, J. Grammes, C. Saari, C. Schulze, G. Yesenosky, P. Aquilina, M. Al-Bataineh, S. Kutalek. Heart Rhythm Society, Annual Scientific Sessions, May 2009, Abstract.
4. Percutaneous coronary sinus lead extraction with prolonged implant duration – a single center experience of 120 leads. J. Grammes, K. Linganathan, I. Ghanma, A. Kohut, R. Mohan, D. Ratliff, J. Ziebeq, C. Saari, G. Yesenosky, M. Al-Bataineh, S. Kutalek. Heart Rhythm Society, Annual Scientific Sessions, May 2009, Abstract.
5. Prognostic significance of retained fibrous casts after percutaneous lead extraction in the setting of infection. V. Mallavarapu, S. Feitell, S. Choi, O. Olaniyan, P. Gorodin, A. Kohut, J. Fontaine, S. Kutalek. Heart Rhythm Society, Annual Scientific Sessions, P01-40, May 2011.
6. Echocardiographic description of retained fibrous casts after percutaneous lead extraction in the setting of infection. V. Mallavarapu, M. Allareddy, S. Feitell, S. Choi, O. Olaniyan, P. Gorodin, A. Kohut, J. Fontaine, S. Kutalek. American Society of Echocardiography, Annual Scientific Sessions, June 2011, Abstract.
7. Superior Vena Cava (SVC) Echocardiography to Predict ICD and Pacemaker Lead Fibrosis: Case Report. S.J. Yakish, A. Narula, B. Foley, A. Kohut, S. Kutalek. DrexelMed Journal, January 2012.
8. Superior Vena Cava (SVC) Echocardiography as a Screening Tool to Predict Cardiovascular Implantable Electronic Device (CIED) Lead Fibrosis: Case Series. S.J. Yakish, A. Narula, B. Foley, A. Kohut, S. Kutalek. International Academy of Cardiology, 17th World Congress on Heart Disease, June 2012.
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