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An Interposed Pad in Open-Chest Echocardiographic Porcine Scans for Mimicking Ultrasound Signal Attenuation of a Human Chest

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SUMMARY

Opening a chest in an experimental echocardiographic animal study eliminates ultrasound signal attenuation by the chest wall. We developed a scanning technique that involves the use of an attenuative pad created from a mixture of urethane and titanium dioxide. The pad was interposed within transmission gel between the transducer face and cardiac surface in open-chest scans in a porcine model. Comparative measurements of left ventricular echogenicity without and with the pad demonstrate that the pad reproducibly causes an ultrasound signal attenuation that closely mimics chest attenuation in clinical transthoracic echocardiographic studies.

Keywords

attenuative pad; open-chest porcine model; ultrasound imaging; ultrasound signal attenuation

INTRODUCTION

Cardiovascular preclinical research has benefited from porcine models depicting heart failure,¹ cardiomyopathy,^{2, 3} or coronary occlusion and myocardial reperfusion,^{4, 5} to name only a few examples. Echocardiographic preclinical porcine studies include transthoracic,⁶ as well as transesophageal and intracardiac,⁷ approaches. However, cardiovascular research, new device development, or procedural training may demand an open-chest study due to the need for animal instrumentation or direct observation of anatomy. In addition, closed-chest transthoracic ultrasound scans in adult domestic pigs do not replicate transthoracic echocardiographic scans in humans due to the differences in pig chest configuration and narrow intercostal spaces⁶ and, therefore, an open-chest setting may be preferred.

The problem with echocardiographic pericardial or epicardial open-chest scans is that they do not include signal attenuation that would otherwise be caused by tissues within an intercostal imaging window during human transthoracic echocardiography. We offer a

solution to this problem by using an attenuative urethane pad, which is interposed within a transmission gel between the transducer face and the heart surface in open-chest porcine studies. The pad induces a signal loss, resulting in a decrease in echogenicity similar to that experienced in clinical transthoracic scans. We document received signal loss in an initial pad assessment in vitro and show the results of left ventricular (LV) echogenicity measured in vivo without and with the attenuative pad interposed.

MATERIALS AND METHODS

Preparation of an Attenuative Urethane Pad

Two-part urethane mold-making rubber (M3115, BJB Enterprises, Tustin, California, USA) was prepared according to the manufacturer's instructions and mixed with fine titanium dioxide particles (AC277737-0010, Fisher Scientific, Pittsburgh, Pennsylvania, USA) at 30% by weight. The mixture was poured into a reusable plastic container from a standard Aquaflex® ultrasound gel pad (Parker Laboratories, Inc., Fairfield, New Jersey, USA) to cure and form a round attenuative pad 85-mm in diameter and approximately 4-mm thick.

Initial Assessment of the Attenuative Pad in Vitro

Figure 1 shows the schematics of the in vitro testing setup. Transmitting and receiving unfocused transducers, 19 mm in diameter, were arranged in a one-way pitch-catch configuration spaced 10 cm apart and aligned by maximizing the received amplitude. The transmitting transducer was driven by a waveform generator (Model 33120A, Agilent Technologies, Inc., Loveland, Colorado, USA) producing 20- μ s 40-cycle bursts of a sinusoidal signal at a 2-MHz frequency. The receiving transducer was connected to an oscilloscope (Model MDO3024, Tektronix, Beaverton, Oregon, USA) and 20 center cycles of each tone burst were averaged for voltage analysis. For attenuation calculation, the received signal amplitude was recorded 3 times without and 3 times with a pad in place. In the latter case, the pad was removed and replaced each time.

Testing Setup with an Open-Chest Porcine Heart

This study was performed in an adult domestic pig and was approved by the Institutional Animal Care and Use Committee. Figure 2A shows the open chest of a pig with the heart suspended on a pericardial cradle and the attenuative pad placed to the side. Figure 2B demonstrates use of the pad. The heart is covered by a layer of an Aquasonic® ultrasound transmission gel (Parker Laboratories, Inc., Fairfield, New Jersey, USA). The pad is placed on the gel layer, and additional transmission gel is applied on top of the pad to ensure signal coupling on both sides of the pad. This setting forms a standoff, with ultrasound signal attenuation defined by the composition and thickness of the pad.

Acquisition of Testing Scans

A commercially available ultrasound machine, Vivid 7 (GE Healthcare, Milwaukee, Wisconsin, USA), was used for image acquisition. An ultrasound probe M4S was set to a 2-MHz fundamental scanning regimen with 54 frames/s and a short-axis projection of the LV at the papillary muscle level. Time gain compensation was adjusted to mid-position at all depth levels, B-mode gain was set to 0 dB, focus was aligned with the bottom of the LV, and

the remaining pre- and postprocessing settings were kept at machine defaults, except for a transmit power. The transmit power was incrementally set at 0 dB, -6 dB, and -10 dB. Testing scans without and with the attenuative pad were acquired at each of these 3 power settings.

Echogenicity Evaluation

Image data were transferred to a dedicated workstation with EchoPAC software, version BT11 (GE Healthcare, Milwaukee, Wisconsin, USA), which included a “Q-Analysis” function. This function was used for assessing echogenicity by an integrated backscatter (ie, relative reflected ultrasonic energy measured in decibels [dB]).^{8–10} Echogenicity changes were evaluated in a manner similar to our previous work.¹¹ However, in the current study, the manually outlined region of interest (ROI) was drawn around the entire LV epicardial circumference at end-systole. Figure 3A presents a scan without the attenuative pad. The left panel shows the systolic LV delineation. Attention was paid to avoid inclusion of high echogenicities at the interface between the epicardium and the surrounding transmission gel. The right panel shows a trace of average LV echogenicities occurring within the delineated ROI, ie, within the LV, at different time points over a period of 3 cardiac cycles. The data are varying between approximately -18 dB and -22 dB. Figure 3B shows the same LV and outline like in Figure 3A, but the echogenicity data trace has been smoothed by a 7-point averaging filter. This was done only to aid in interactive selection of the 3 most stable cardiac cycles of unprocessed data (Figure 3A) for echogenicity analysis. Instability in data was typically caused by a respiratory motion or an accidental displacement (slippage) of the transducer on the pad. Figures 4A and 4B are analogous to Figures 3A and 3B, but present an LV scan and data obtained with the attenuative pad. Notice that the LV appears darker and the unprocessed echogenicity trace values (Figure 4A) range roughly between -25 dB and -31 dB.

Data Analysis

During the preliminary evaluation in vitro, peak-to-peak voltages (V_{pp}) were measured at the receiving transducer (Figure 1). Two average values, one from 3 measurements without a pad and the other after 3 repeated pad interpositions, were obtained. Signal loss (dB) was calculated as $20 \times \log ([V_{pp} \text{ with pad}]/[V_{pp} \text{ without pad}])$ and represented attenuation by a single trip.

In the test setting in vivo, echogenicity (dB) traces were obtained without and with the pad at 0, -6, and -10 dB transmit power settings. Mean $\pm$ standard deviation (SD) and median values were calculated from traces of echogenicity after LV delineation in EchoPac. Typically, the traces encompassed 3 cardiac cycles of unprocessed echogenicity data for analysis. Overall average echogenicity changes without and with the pad (pooled from all 3 power settings) were calculated as well and represented attenuation by a round-trip imaging signal. The analyses were performed independently by 2 observers (M.B. and M.K.).

RESULTS

Urethane Pad – Mechanical Properties and in Vitro Signal Attenuation

The titanium dioxide urethane pads were quite compliant, with a durometer Shore hardness of 15A (<https://www.smooth-on.com/page/durometer-shore-hardness-scale>). Based on our experience from repeated preliminary experiments, these pads needed to be about 4-mm thick to induce approximately 4 dB of signal attenuation at a 2-MHz frequency in a single-trip testing setup in vitro (Figure 1). A-lines of radiofrequency signals sampled without and with the attenuative pad interposed are shown in Figure 1 and demonstrate that the transmitted signal remained without noticeable noise or dispersion after attenuation. The attenuative pad used in the current study was exactly 3.7-mm thick. The average measurements at the receiving transducer were 3.033 Vpp without the pad and 1.870 Vpp with the pad, resulting in a signal change of -4.2 dB in a single trip at 2-MHz frequency. Therefore, the pad attenuation coefficient was $4.2/0.37/2 = 5.66$ dB/cm/MHz. We also measured density and propagation speed of the pad to calculate its impedance, which was 1.66×10^6 kg/m²/s.

In Vivo Echogenicity Analysis

Table 1 and Table 2 show the mean and median echogenicity values, respectively, measured by the 2 observers (M.B. and M.K.) in round-trip scans in vivo. At transmit power settings of 0, -6 , and -10 dB, insertion of the attenuative pad induced changes in mean and median echogenicity measurements ranging from -5.71 to -8.85 dB. However, the average mean and median echogenicity changes were between -7.1 and -7.7 dB. SD values of means in Table 1 resulted in an approximately 5% coefficient of variation in averaged echogenicities, irrespective of an observer and absence or presence of the attenuative pad.

DISCUSSION

With this study, we show that a custom attenuative pad can be used in open-chest preclinical ultrasound imaging investigations in large animals to mimic attenuation typical for a human chest in clinical transthoracic scans. In general, chest attenuation ranges from -5 to -15 dB.¹² We present an attenuative pad that is made of a urethane and titanium dioxide mixture. At 2-MHz ultrasound signal frequency, this pad changes LV echogenicity by approximately -7.4 dB (an overall average from Tables 1 and 2). Such value fits well within an attenuative range of the human chest wall in clinical transthoracic scans.

The porcine heart was placed on a pericardial cradle during the experimental study. One purpose was to minimize cardiac and respiratory motions and their undesired effects on acoustic coupling intermediated by a gel layer between the transducer face and the attenuative pad and between the pad and epicardial surface. However, these effects could not be entirely eliminated and alterations in acoustic coupling during testing scans likely contributed to variations in echogenicity change reported in Tables 1 and 2. Other probable factors influencing variability in the measured echogenicity changes included an insonation angle by the transducer and signal transformation into imaging data by the ultrasound machine. Nonlinearities in signal processing within the ultrasound machine and along the

ultrasound transmission path can explain echogenicity changes at different testing levels of the transmit power (Tables 1 and 2). Besides the general range of chest attenuation in clinical transthoracic scans, ultrasound signal loss, when scanning certain cardiac segments, can reach -40 dB.¹² In this context, variations in echogenicity changes induced by interposing the attenuative pad presented in Tables 1 and 2 are deemed to be within a relatively narrow range.

When and Why to Use an Attenuative Pad in Preclinical Open-chest Animal Studies

One reason for choosing an open-chest setting in pigs or other large animals is to ensure high-quality, unobstructed ultrasound images of a cardiovascular system if transesophageal, intracardiac, or intravascular scans are not an option. However, in a variety of investigative studies or procedural training sessions, the reason for chest opening is instrumentation or observation of the cardiovascular system. In these cases, recordings of clinically “realistic” (ie, attenuated) scans are highly desirable. While closing and evacuating the chest may restore basic preoperative anatomical configuration, the acoustic tissue interfaces are, based on our experience, considerably affected. In chronic studies, accelerated fibrosis (healing reaction) adds to transthoracic acoustic alterations. Another reason for utilizing open-chest cardiac scans with the attenuative pad is that transthoracic ultrasound imaging in a pig, even with an intact chest, is technically challenging. The intercostal spaces are narrow, thus limiting the scanning window, and the predominantly anteroposterior prolongation of a chest cross-sectional shape, along with an anteroposterior orientation, causes the heart long-axis to conform to the 4-legged stance of a pig, but does not closely replicate the anatomy (and thus standardized projections) of a human chest.⁶

Conceivably, attenuation of the chest could also be simulated during open-chest pericardial or epicardial scans by intentionally reducing the transmit power. However, such an approach would not replicate the interfaces between the transducer face and the “chest wall” (simulated by the pad) and between the pad and a pericardial or epicardial surface. Moreover, the mixture of urethane and titanium dioxide (ie, material for pad preparation) produces a texture pattern in echocardiographic scans similar to that of a soft tissue, whereas pad thickness selected during manufacturing controls the amount of signal loss at a given ultrasound frequency. Finally, the pad and a layer of transmission gel form a standoff, as can be seen in Figure 2B. Based on our practical experience, such a standoff can position the transducer face far enough apart from the surface of the heart to simulate separation of the transducer from the heart by the chest wall. The standoff also provides enough separation of the transducer face from the cardiac surface to prevent near-field scans, in which some parts of the heart could be located too close to the transducer to be usefully imaged.

Practical Use of the Attenuative Pad

We used an attenuative pad in an open-chest study for the first time a decade ago.¹³ Initially, we utilized urethane material from Smooth-On, Inc (Macungie, Pennsylvania, USA) with no titanium dioxide additive. The resulting pads were very durable and lasted for several years. But the pads were very stiff and had to be made thicker to induce the same level of attenuation as the pads presented in this study. The pads used in the current study, however, have a shorter lifetime. After 2 months of use in 12 extensive open-chest porcine studies

(approximately 20–30 insertions per study), the pads developed multiple cracks. Therefore, our future plan is to experiment with a mixture of the Smooth-On urethane and titanium dioxide particles to achieve an optimal combination of durability, thickness, flexibility, attenuation, and appearance in ultrasound images.

While use of the interposed attenuative pad is straightforward, careful attention has to be paid to preventing small air bubbles from forming in the transmission gel beneath the pad (due to cyclic respiratory and cardiac motions) and above the pad (due to transducer motion when seeking the desired projection). Based on our practical experience, it is advisable to replace the gel approximately every 20 minutes during the course of ultrasound imaging with the pad. An occurrence of air bubbles in the transmission gel inside a container (we pour the gel directly from a 1-gallon container) can be minimized prior to the study by warming the container so that the gel content liquefies and the bubbles float upward and break. The pad-heart transition forms a well matched interface as the measured acoustic impedance of the pad ($1.66 \times 10^6 \text{ kg/m}^2/\text{s}$) is very close to the impedance of the heart ($1.64 \times 10^6 \text{ kg/m}^2/\text{s}$).¹⁴

Recently, we came up with a simplified and practical use for the current thin and flexible attenuative pad. We cut out a piece of the pad so that it matched the shape of the transducer face. A thin layer of a transmission gel was then introduced on both sides of the pad to ensure acoustic coupling. Covering the pad and the transducer by a stretched latex ultrasound probe cover (Sheathing Technologies, Inc, Morgan Hill, California, USA) secured the pad in place. The consecutive photos in Figure 5 demonstrate placement and use of such a pad. We anticipate that this arrangement will protect the pad, thus extending its lifetime, and minimize the issue of air bubbles forming in a layer of transmission gel applied below and above the regular attenuative pad.

Limitations

The use of a pad can simulate the amount of signal attenuation by soft tissue between ribs, but does not replicate a limited intercostal scanning window. This could be a methodological issue in very specialized, perhaps instrument development, studies.

Another practical limitation is that when both the interposed attenuative pad and the transmission gel layer underneath it are inadvertently pushed by the hand-held ultrasound probe, the resulting physical load on the heart could restrict or alter its motion. Opening the chest and dissecting the pericardium already impact cardiac motion. Nevertheless, in studies where assessment of cardiac function is the investigative goal, attention needs to be paid to this possibly confounding effect on cardiac mechanics. Using the pad cutout directly attached to the transducer, as shown in Figure 5, is expected to alleviate this problem.

Unlike in a typical tissue characterization study, in which the ROI represents a selected tissue sample, such as in myocardial analysis of viability⁸ or carotid artery analysis for a presence of atherosclerotic changes,¹⁵ we employed a static (ie, non-tracking) manual delineation of the entire LV in a short-axis view. The primary reason for avoiding the selective ROI was that echogenicity values produced by such an ROI can be substantially confounded by extreme local echogenicities that have unpredictably moved into the selective

sample during cardiac and respiratory movements, despite tissue tracking by the selective ROI. In the current study, using the entire LV in a short-axis view as the ROI for echogenicity analysis produced reproducible results, as documented by the low coefficient of variation.

Clinical Relevance

Configuration of a pig chest is different from that of a human, which arguably makes a closed-chest model problematic. However, open-chest ultrasound scans made directly on the cardiac surface unrealistically lack attenuation and the transducer face is located too close to the heart. The attenuative pad can replicate ultrasound signal loss in transthoracic scans in humans, avoid unnecessary challenges of transthoracic scans in pigs, and provide a standoff that facilitates a proper distance of the transducer face from the cardiac surface. Scans with the attenuative pad do not add any marked expense and are practical for preclinical research, instrument development, and procedural training studies.

CONCLUSION

An interposed attenuative pad represents a practical solution for mimicking human chest attenuation in open-chest experimental studies in large animals.

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Figure 1.

In vitro testing setup. Transmitting and receiving unfocused transducers are immersed in degassed water 10-cm apart and the tested pad is interposed between them. The waveform generator outputs 40-cycle bursts of a 2-MHz sine signal that is 20-dB amplified and drives the transmitting transducer and also produces a synchronization signal that triggers the oscilloscope (upper panel). The peak-to-peak voltage (V) of the radiofrequency signal obtained from the receiving transducer is assessed in the oscilloscope on a microsecond (μ s) time scale in the presence and absence of the pad (lower panel).

Figure 2.

In vivo open-chest testing setup. **A**, An attenuative pad is prepared next to a beating pig heart exposed on a pericardial cradle. A scan without a pad is performed first through a layer of transmission gel (not shown). **B**, The attenuative pad is interposed between the heart surface and transducer face. The transmission gel ensures acoustic coupling.

Figure 3.

Nonattenuated left ventricular (LV) scan and echogenicity data selection. **A**, The LV is outlined inside of the epicardium in a short-axis view. The delineated area represents the region of interest (ROI). The right panel shows traces of unprocessed echogenicity values (the portion delimited by the dashed lines was used for analysis). **B**, Identical LV scan and outline as in (A), but the trace is smoothed with a 7-point averaging filter to ease an interactive selection of a 3-cardiac-cycle-long segment of the most stable echogenicity data delimited by the dashed lines. The selected segment then defines the corresponding segment of unprocessed data used for calculating mean, SD, and median values.

Figure 4.

Attenuated left ventricular (LV) scan. **A**, The scan is similar to that in Figure 3, but an attenuative pad has been interposed between the heart and the transducer, and is visible in the LV ultrasound image (arrowhead). The right panel shows a segment of unprocessed traces (delimited by the dashed lines) of echogenicity data used for analysis. Notice that the data are at a different dB level as compared to those in Figure 3. **B**, Identical LV scan and outline as in (A). The smoothed trace aids in interactive selection of 3 cardiac cycles of the most stable unprocessed data (dashed lines).

Figure 5. Attenuative pad placed directly on the transducer face. The consecutive photos show placement of a pad that matches the area of the transducer face (upper panels), a transducer prepared for attenuated scans (middle panel), and an attenuated scan through the cardiac anterior free wall in an open-chest pig (bottom panel).

Table 1Mean $\pm$ SD of LV Echogenicity Without and With Attenuative Pad

Observer	Transmit Power (dB)	Mean $\pm$ SD LV Echogenicity (dB)		Echogenicity Change (dB)	Average Echogenicity Change (dB)
		Without Pad	With Pad		
M.B.	0	-19.915 $\pm$ 0.788	-28.586 $\pm$ 0.958	-8.67	-7.1
	-6	-20.164 $\pm$ 0.660	-27.083 $\pm$ 1.419	-6.92	
	-10	-21.974 $\pm$ 1.208	-27.687 $\pm$ 1.783	-5.71	
M.K.	0	-19.912 $\pm$ 0.981	-28.604 $\pm$ 1.204	-8.69	-7.4
	-6	-19.991 $\pm$ 0.844	-27.473 $\pm$ 1.558	-7.48	
	-10	-21.646 $\pm$ 1.150	-27.751 $\pm$ 1.683	-6.10	

LV, left ventricular; SD, standard deviation

Table 2

Median of LV Echogenicity Without and With Attenuative Pad

Observer	Transmit Power (dB)	Median LV Echogenicity (dB)		Echogenicity Change (dB)	Average Echogenicity Change (dB)
		Without Pad	With Pad		
M.B.	0	-19.929	-28.774	-8.85	-7.3
	-6	-20.146	-27.105	-6.96	
	-10	-21.868	-27.865	-6.00	
M.K.	0	-20.324	-29.019	-8.70	-7.7
	-6	-19.818	-27.611	-7.79	
	-10	-21.698	-28.273	-6.58	
LV, left ventricular					