Pulse Wave Imaging of Normal and Aneurysmal Abdominal Aortas In Vivo

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Abstract—The abdominal aortic aneurysm (AAA) is a common vascular disease. The current clinical criterion for treating AAAs is an increased diameter above a critical value. However, the maximum diameter does not correlate well with aortic rupture, the main cause of death from AAA disease. AAA disease leads to changes in the aortic wall mechanical properties. The pulse-wave velocity (PWV) may indicate such a change. Because of limitations in temporal and spatial resolution, the widely used foot-to-foot method measures the global, instead of regional, PWV between two points at a certain distance in the circulation. However, mechanical properties are nonuniform along the normal and pathological (e.g., the AAA and atherosclerosis) arteries; thus, such changes are typically regional. Pulse-wave imaging (PWI) has been developed by our group to map the pulse-wave propagation along the abdominal aorta in mice in vivo. By using a retrospective electrocardiogram (ECG) gating technique, the radio-frequency (RF) signals over one cardiac cycle were obtained in murine aortas at the extremely high frame rate of 8 kHz and with a field-of-view (FOV) of $12 \times 12 \text{ m}^2$. The velocities of the aortic wall were estimated using an RF-based speckle tracking method. An Angiotensin II (AngII) infusion-based AAA model was used to simulate the human AAA case. Sequences of wall velocity images can noninvasively and quantitatively map the propagation of the pulse wave along the aortic wall. In the normal and sham aortas, the propagation of the pulse wave was relatively uniform along the wall, while in the AngII-treated aortas, the propagation was shown to be nonuniform. There was no significant difference ($P > 0.05$) in the PWV between sham (4.67 ± 1.15 m/s, $n_2 = 3$) and AngII-treated (4.34 ± 1.48 m/s, $n_2 = 17$) aortas. The correlation coefficient of the linear regression was significantly higher ($P < 0.005$) in the sham aortas ($0.89 \pm 0.03$, $n_1 = 5$) than in the AngII-treated ones ($0.61 \pm 0.15$, $n_1 = 17$). The wall velocities induced by the pulse wave were lower and the pulse wave moved nonuniformly along the AngII-treated aorta ($P < 0.005$), with the lowest velocities at the aneurysmal regions. The discrepancy in the regional wall velocity and the nonuniform pulse-wave propagation along the AngII-treated aorta indicated the inhomogeneities in the aortic wall properties, and the reduced wall velocities indicated stiffening of the aneurysmal wall. This novel technique may thus constitute an early detection tool of vascular degeneration as well as serve as a suitable predictor of AAA rupture, complementary to the current clinical screening practice.

Index Terms—Abdominal aortic aneurysm (AAA), pulse wave, speckle tracking, ultrasound.

I. INTRODUCTION

The abdominal aortic aneurysm (AAA) is the most common form of aneurysm and a frequently lethal disease of the older population, with more than 13,000 deaths in the United States annually [1]. The incidence of AAAs is between 1.3% and 8.9% in men and between 1.0% and 2.2% in women [2].

The most standard diagnostic technique for the detection of AAA is abdominal ultrasound or computed tomography (CT) [2], [3]. The risk of rupture is highest when the transverse diameter of the aneurysm reaches 5 or 5.5 cm [4]–[6]. However, the maximum diameter criterion is not reliable and does not have a physically sound theoretical basis [3]. Most AAAs are asymptomatic and, therefore, rupture can occur without a warning, e.g., without a significantly increased diameter [7], [8]. An effective screening technique is therefore warranted. In addition, an AAA-diameter increase beyond 5 cm correlates with only 40% chance of aneurysm rupture [9]. This poses a serious dilemma to surgeons that need to operate on AAAs only when the risk of rupture is higher than the risk of the procedure itself [3]. However, the rupture risk cannot be properly predicted given the current methods available in the clinic.

Aortic stiffness has been indicated as an early predictor of cardiovascular mortality, primary coronary events, and fatal stroke [10]–[15]. The stiffness of the arterial wall is mainly determined by the matrix components of the wall, i.e., the elastin, collagen, and smooth-muscle cells. Changes in the composition and structure of the wall will alter its stiffness [16]. Various vascular diseases including AAAs are known to change the tissue mechanical properties. Noninvasive methods to evaluate the mechanical properties of the AAA wall have been investigated by several groups. MacSweeney et al. measured the distensibility of the aorta using M-mode ultrasound and found increased stiffness in aneurysmal vessels [17]. Long et al. measured compliance of the abdominal aortic wall of AAA using a tissue Doppler imaging system [18], [19]. They found a significant increase in segmental dilation and compliance with increased AAA diameter. Truijers et al. evaluated AAA patients using CT and calculated the wall stress using a finite-element method [20]. Wall stress was concluded to be significantly higher in the ruptured than in the asymptomatic aneurysms, whereas no differ-

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ences were observed in the transverse diameter between asymptomatic, symptomatic, and ruptured aneurisms. Brekken et al. estimated circumferential strains in AAAs from sequences of ultrasound B-mode images [21]. They found that regional strain values might exceed the circumferential average strain significantly, and the strain showed no apparent correlation with the diagnosed diameters.

Pulse-wave velocity (PWV) is typically used for estimating the stiffness of arteries [22]–[25]. Pulse waves are flow velocity, pressure, and diameter waves generated at the ejection phase of the left ventricle [22]–[25]. Their propagation speeds and patterns are related to the underlying vascular mechanical properties (e.g., arterial stiffness). In a straight elastic tube containing a nonviscous liquid, the disturbances in flow velocity, pressure, tube diameter, or wall displacement (and wall velocity), propagate as waves along the tube, at a certain velocity [26]. The Young’s modulus of the tube is related to the wave velocity through the Moens–Korteweg equation as follows [22]:

\[ c = \sqrt{\frac{E h}{2 R \rho}} \]  

(1)

where \( c \) is the wave velocity, \( E \) is the Young’s modulus of the conduit wall, \( h \) is the wall thickness, \( \rho \) is the density of the wall, and \( R \) the inner radius of the tube.

The PWV can be measured through a flow velocity wave, pressure wave or diameter wave [22]. In the conventional foot-to-foot method, the waveforms at the two sites (typically, at the common carotid and femoral arteries) are recorded simultaneously, or sequentially, using registration with a simultaneously recorded electrocardiogram (ECG) [25]. The PWV is calculated as the distance between two measurement points divided by the time shift of the waveforms from these two points [22]–[25].

Despite the simple definition of PWV, some problems still remain, which limit the interpretation of available findings and the general applicability of the PWV measurement [27]–[29]. The accuracy of PWV measured from two separate points suffers from errors of distance measurements and/or time-delay measurements [29]. It is difficult to measure small time shifts, because the temporal resolution is not high enough for fast pulse-wave propagation. Usually, a large time shift (e.g., \( >10 \) ms) over a relative larger distance (e.g., a few centimeters) is thus needed for reliable measurements. Therefore, the PWV measured represents the average value between two measurement sites. In addition, the distance measurement is based on the assumption that the arterial wall does not change its geometry between the two measurement points, which may not always hold [25]. Large errors in the distance measurement may also increase when the two measurement points are several centimeters away because the vessel between these two points may not necessarily be uniform or straight, especially in the presence of disease. Moreover, these points may not always be along the line of travel of the pulse wave [24]. In the carotid and femoral arteries, for example, the pulse wave travels in opposite directions. A method with higher temporal and spatial resolution is thus needed in order to calculate the regional PWV.

Mouse models are becoming increasingly popular in cardiovascular research. However, the small size of the mouse abdominal aorta (1–2 cm in length and approximately 1 mm in diameter) and high PWV (e.g., \( 2.2–8.5 \) m/s [30]) require an imaging modality with both high temporal and spatial resolution in order to measure the regional PWV and image the pulse-wave propagation. For a given PWV of 4 m/s and an aortic length of 12 mm (i.e., the image width in this study) in mice, it takes only 3 ms for the pulse wave to travel and, therefore, a frame rate of at least 333 Hz is required to capture the wave. The higher the frame rate is, the more images are capable to depict the pulse wave, and the more detailed spatio-temporal information on the propagation can be obtained.

A noninvasive imaging method for visualizing the pulse wave and calculating the regional PWV in the aorta may contribute to a future screening test to reduce the overall morbidity and mortality. Ultrasound allows noninvasive measurements of PWV, and has been an area of recent research interest [30]–[36]. High-frequency, small animal ultrasound systems have also recently become commercially available. To overcome frame-rate limitations in small animal applications, retrospective ECG gating or prospective ECG triggering has been used [37]–[41]. Williams et al. evaluated the feasibility of noninvasively measuring PWV in the mouse carotid artery using a high-resolution Vevo 770 system (VisualSonics Inc., Toronto, ON, Canada) [40]. Our group recently developed an ultrafast radio-frequency (RF) data acquisition system based on the Vevo 770 system and the ECG gating technique [39]. The RF signals of the murine myocardium and aorta in a full field-of-view (FOV) could be obtained at an extremely high frame rate (8000 frames/s or, 8 kHz), and used to estimate the small motion across the murine left ventricle and abdominal aorta [39], [41]–[43].

Using this system with both high temporal and spatial resolution, pulse-wave imaging (PWI) has previously been developed to visually map the pulse-wave propagation, from the proximal (close to the heart) to the distal (close to the renal junction) regions along the aortic wall (<12 mm) in mice [42], [43]. In the previous study, an AAA model using the perirterial application of calcium chloride (CaCl\(_2\)) was used to investigate the capability of PWI for detecting diseases [42], [43]. However, that model might not be very close to the human AAAs because an expansion of the aortic cross section was not observed [42], [43]. In this paper, we used a more suitable AAA mouse model, i.e., the Angiotensin II (AngII) model. This model is known to induce aneurysms with many characteristics of the diseased tissue in humans including luminal dilation, degeneration of the medial wall, thrombosis, and inflammation [44], [45].

II. METHODS

A. Animal Preparation

Wild-type C57BL/6 male mice (7–10 months old) were obtained from the Jackson (Bar Harbor, ME) and Taconic (Germantown, NY) Laboratories. All procedures were approved by the Institutional Animal Care and Use Committee of St Luke’s–Roosevelt Hospital Center and Columbia University.
After intraperitoneal administration of 125 mg/kg tribromoethanol (Sigma-Aldrich Corp., St. Louis, MO), a subcutaneous osmotic minipump (Alzet model 2004, Duract Corp., Cupertino, CA) was implanted into the mice to deliver a slow release of AngII (1.44 mg/kg/day) or 1 M phosphate buffered saline (sham). On day 28 (unless otherwise stated), the mice were anesthetized with an inhaled mixture of 1%–2% isoflurane (AErrane, Baxter Healthcare Corp., Deerfield, IL) and oxygen (Tech Air of Connecticut, Inc., White Plains, NY) by using an isoflurane vaporizer (Model 100, SurgiVet, Inc., Waukesha, WI). The hair over the abdomen and thorax was removed. The mice were placed supine on a heating platform (THM100, Indus Instruments, Houston, TX) to maintain a constant body temperature (approximately 37 °C). The ultrasound probe was placed on the murine abdomen using degassed ultrasound gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, NJ) as a coupling medium. Care was taken to avoid the formation of air bubbles in the gel.

The criterion for an AAA in this study was a diameter increase in the suprarenal region higher than 30% of the diameter in the proximal (i.e., closer to the thoracic aorta) region based on the measurement using B-mode images. Five sham mice infused with saline were used as a control and these mice did not develop any AAAs. Thirty mice were treated with AngII, among which 17 mice developed AAAs. The aneurysms were confirmed with the histopathology and the dilation of the suprarenal aorta. In this paper, only the aneurysmal aortas were considered in the AngII-treated group in order to reduce the effects of different responses of the aorta to the AngII infusion. Two aortas showed nonsignificant diameter increase (<50% of the original diameter in the proximal region) while the remaining 15 aortas showed significant diameter increase (>50% of the original diameter in the proximal region). These two groups were referred to as AngII D- and AngII D+, respectively.

On one occasion, the aorta was found to rupture five days after the AngII treatment and was imaged during rupture. The results of this mouse before and after rupture were included as the normal and ruptured cases, respectively.

B. Data Acquisition

The high-frame-rate data acquisition system previously developed [39] was used in the in vivo experiments. A 30-MHz ultrasound probe (RMV-707B, VisualSonics Inc., Toronto, ON, Canada) was placed on the murine abdomen. A longitudinal (long-axis) view of the abdominal aorta was obtained and efforts were made to align the radial direction of the majority of the aorta with the axial direction of the ultrasound beams. In each case, the FOV was equal to 12 × 12 mm², the axial resolution was equal to 55 μm, and the lateral resolution was equal to 115 μm.

In the EKV (ECG-based kilohertz visualization) mode provided by the imaging system (Vevo 770, VisualSonics Inc., Toronto, ON, Canada), the single-element transducer operated on a line-by-line basis. The transducer transmitted and received at a pulse-repetition frequency (PRF) of 8 kHz. The data acquisition was triggered by a synchronization signal on the RF signals, which indicated the time of pulse transmission of the transducer. The ECG was acquired using the electrode leads available on the heating mouse platform. A two-channel, 14-bit waveform digitizer (CompuScope 14200, Gage Applied Technologies Inc., Lachine, QC, Canada) was used to acquire the RF signals and the associated ECG simultaneously, at 160 MHz and 8 kHz, respectively. Each acquisition lasted approximately 7 min. After data acquisition, the acquired RF signals were gated between two consecutive R-wave peaks to reconstruct the RF frame sequence for a complete cardiac cycle at the extremely high frame rate of 8 kHz [39], [41].

C. Data Processing

The incremental (i.e., between consecutive RF frames), axial (i.e., along the direction of ultrasound propagation) pulse-wave induced wall displacement were estimated offline using a 1-D normalized cross-correlation technique on the RF signals obtained [39], [41]–[43]. The window size was equal to 240 μm with a 90% overlap. The rigid motion induced by respiration was removed by subtracting the motion of the surrounding tissue from the motion of the aorta. The velocity of the aortic wall was calculated as the incremental displacement (i.e., instantaneous motion) divided by the time interval.

Fig. 1 shows a schematic of the propagation of a pulse wave along the abdominal aorta over a length of 12 mm. The axial direction of the ultrasound beam is parallel to the radial direction of the abdominal aorta in the long-axis view. At the time point of \( t_{PW} = 0 \), the pulse wave reaches the longitudinal position \( x_{PW} = 0 \) along the aorta. At the time point of \( t_{PW} = t_1 \) and \( t_2 \), the pulse wave reaches the longitudinal position \( x_{PW} = x_1 \) and \( x_2 \), respectively. The position \( x_{PW} \) and time \( t_{PW} \) of the pulse wave are determined from the spatio-temporal information of the estimated wall velocity \( u \) (upward arrows in Fig. 1).

The estimated wall velocities were color-coded and overlaid onto the 2-D grayscale ultrasonic images (i.e., B-mode images). The sequence of wall velocity images were generated over the entire cardiac cycle and formed a ciné-loop. The forward pulse wave was clearly visualized, which propagated from the proximal (heart) to the distal (kidney) positions of the suprarenal abdominal aorta. The propagation pattern of the pulse wave was

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Fig. 1. Illustration of the pulse wave propagation along the abdominal aorta and the pulse wave imaging field of view.
qualitatively examined based on the PWI ciné-loops and images. For clarity purposes, only the wave on the anterior [i.e., nearest to the transducer (top)] wall was shown. PWI can also estimate the wave on the posterior wall (not shown).

The extent of the aortic wall was obtained though manual tracing on the ultrasound (B-mode) image by a trained observer. The velocity transition of the aortic wall segment at a specific beam position was plotted as a function of the time lapsed after the R-wave. The profiles along the imaged aorta are shown in a waterfall plot, or 2-D image, indicating the spatio-temporal variation of the pulse-wave propagation.

On the 2-D spatio-temporal images, the peak velocity and the time corresponding to the foot (onset of upstroke) of the pulse were both obtained for each beam position. The peak velocities were detected within a small, preselected time interval (typically <5 ms), so as to reduce the effects of noise and reflected waves. In order to further reduce the effects of the reflected waves on the PWV estimation, the foot of the waveform was used as the characteristic point of the pulse occurrence [22]. The foot of the wall velocity pulse was defined as the time-point, at which the velocity attained a value equal to 25% of the peak velocity, as suggested by Hocott et al. [46]. Compared to the peak of the pulse previously used [42], [43], the foot was less sensitive to the reflected wave and noise due to its independence of the reflected wave. In addition, the foot was easier to localize in the case of reduced wall velocities.

The time of peak velocity occurrence (i.e., the dependent variable) was plotted as a function of distance (i.e., the independent variable) from the proximal edge. Linear regression was applied to the time-distance plot. The PWV was calculated as the invariable) from the proximal edge. Linear regression was applied for the PWV estimation, the foot of the waveform was defined as the time-point, at which the velocity attained a value equal to 25% of the peak velocity, as suggested by Hocott et al. [46]. Compared to the peak of the pulse previously used [42], [43], the foot was less sensitive to the reflected wave and noise due to its independence of the reflected wave. In addition, the foot was easier to localize in the case of reduced wall velocities.

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Throughout this paper, the PWI was defined as the method of visually mapping the propagation of the pulse wave along the aorta. PWI images thus denoted either the 2-D color-coded wall-velocity images or the 2-D spatio-temporal images.

D. Histopathology

At the completion of each experiment, the mice were sacrificed and surgically operated on their ventral surface to expose both the entire abdomen and thoracic cage. Latex injection was given through the left ventricle to distend the aorta (1 cc). The aorta was then found to have ruptured, through visible hemorrhage under gross pathology. It should be noted that, in this case, it is difficult to detect the aneurysm or rupture from the B-mode images.

As indicated by the solid arrows, the propagation of the pulse wave in the AngII D+ aorta is similar to that in the normal aorta (Fig. 2).

The sequence of PWI images in an AngII-treated aorta with a nonsignificant diameter increase (i.e., the AngII D+ aorta) is shown in Fig. 4. In Fig. 4(d), the arrow indicates the suspicious region detected by the PWI image, i.e., the region having significantly lower velocity than the proximal region of the aorta. Compared to the normal (Fig. 2) and sham (Fig. 3) cases, the average wall velocity of this AngII D+ aorta is slightly lower.

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Fig. 2. The sequence of PWI images every 1.25 ms (i.e., 10 frames) in a normal aorta. The wall velocities are color-coded and overlaid onto the B-mode image. Positive velocities (in red) represent upward motion, while negative velocities (in blue) represent downward motion (the same below). The time after the R-wave of ECG is shown in the title of each figure. Only the velocities on the anterior wall of the aorta and the surrounding tissue are shown for better visualization. The solid arrows indicate the propagation of the pulse wave from the proximal to the distal site (a)–(d).

Fig. 3. The sequence of PWI images every 1.25 ms in a sham aorta. Only the velocities on the anterior wall of the aorta and the surrounding tissue are shown for better visualization. The solid arrows indicate the propagation of the pulse wave from the proximal to the distal site (a)–(d). The histology (40×) of the transverse section of a sham aorta (H&E stain) is also shown in (e).

Fig. 4. The sequence of PWI images every 1.25 ms in an AngII-treated aorta, where the diameter change is not significant (>50%). The arrow indicates the region undergoing reduced wall velocities (d).

Fig. 5. The sequence of PWI images every 1.25 ms in an AngII-treated aorta, where the diameter change is significant (>50%). The arrow indicates the region undergoing reduced wall velocities (d). The histology (40×) of an AngII-treated aorta is also shown in (e) and (f), with H&E and EVG stain, respectively. The thin black line at five o’clock of the histology is due to the overlap of the aortic segments in the examination.

C. Pulse Wave Velocity Estimation

From the spatio-temporal images of pulse-wave propagation of the normal case [Fig. 7(b)], the time occurrence of the foot of the pulse wave are obtained at each beam position and shown in a scatter plot in Fig. 8(a). Linear regression was then performed [Fig. 8(a)]. The scatter plot and linear regression line are also shown separately in Fig. 8(b). The vertical axis represents the relative time of the pulse wave, $t_{pW}$, i.e., the time of peak velocity occurrence relative to the arrival time at the

the velocity distribution. The aortic diameters were obtained by manually tracing both the anterior and posterior walls. The spatio-temporal imaging of the sham aorta [Fig. 7(c)] is similar to the normal case [Fig. 7(b)] undergoing uniform propagation of the pulse wave. After AngII treatment, however, the velocity of the aortic wall drops [Fig. 7(d)–(f)]. The velocity reduction in the AngII D+ case [Fig. 7(d)] is higher than in the AngII D- case [Fig. 7(c)]. The rupture case reveals the lowest overall wall velocity out of the five cases. In addition, in all three AngII-treated aortas [Fig. 7(d)–(f)], the wall velocities in the aneurysmal region are the lowest in amplitude while those in the proximal region are the largest; thus, consistent with the previous findings of Figs. 4–6.
Fig. 6. The sequence of PWI images every 1.25 ms in an AngII-treated aorta where is found to rupture. The arrow indicates the region undergoing reduced wall velocities (d). The histology (40×) of the ruptured aorta (Trichrome stain) is also shown in (e), with an arrow indicating the disruption of the elastic lamina.

Fig. 7. Spatio-temporal imaging of the pulse-wave propagation. (a) Waterfall plot of the velocity variation with time and longitudinal position of the aorta. The aorta corresponds to the normal case of Fig. 2. (b)–(f) The 2-D spatio-temporal the velocity variation corresponding to the aortas shown in Figs. 2–6, respectively. In (d) and (e), the aortic diameter outline is also shown on the left side of the spatio-temporal plots.

Fig. 8. Linear regression of the pulse-wave propagation and estimation of the PWV. (a) is the spatio-temporal imaging of pulse-wave propagation in a normal aorta shown in Fig. 7(b). The time when the peak velocity occurs is depicted for each longitudinal position of the aorta. A linear regression is applied on the time of the foot of the pulse versus the longitudinal position of the aorta. The PWV is estimated as the inverse of the slope of the linear regression. The dashed rectangle in (a) indicated the small, preselected time interval within which the local peak velocity was detected at each longitudinal position. (b)–(f) show the linear regression applied to the aortas shown in Figs. 2–6, respectively. The estimated PWV and the correlation coefficients (r) of linear regression are also shown in (b)–(f).

The linear regression fit was applied to the plot of $t_{PW}$ (dependent variable) versus $x_{PW}$ (independent variable). The PWV along the aorta is estimated as the inverse of the slope of the linear regression fit and is equal to 6.09 m/s in the normal case [Fig. 8(b)]. The linear regression has a high correlation coefficient (r = 0.90). In other words, the propagation of the pulse wave is relatively uniform along the aortic wall. Fig. 8(c) shows the linear regression fit of the pulse wave propagation for the sham aorta. The results of the sham aorta [Fig. 8(c)] are similar to those of the normal case [Fig. 8(b)], with a relatively high correlation coefficient (r = 0.90).

Fig. 8(d)–(f) shows the scatter points and linear regression fit of all three AngII-treated aortas. The AngII-treated aortas [Fig. 8(d)–(f)] exhibit a lower correlation coefficient (r). In addition, in the AngII D- [Fig. 8(d)], AngII D+ [Fig. 8(e)] and the ruptured [Fig. 8(f)] aortas, the correlation coefficient (r) steadily decreases from 0.85 to 0.80 and 0.67, respectively. This implies that the propagation of the pulse wave, and thus the underlying wall, is nonuniform across the entire FOV. Nonuniformities are due to, and may thus indicate, abnormalities.
**D. Statistical Results**

Statistical analysis were performed on five ($n = 5$) sham and seventeen ($n = 17$) AngII-treated aortas. A Student’s $t$-test was used to verify the statistical significance of the difference between the sham (control) and treated aortas. Fig. 9(a) and (b) shows the average PWV and linear regression correlation coefficient ($\rho$) of the sham (control) and AngII-treated aortas within the entire FOV (12 mm). There is no significant difference ($p > 0.05$) in the PWV between the sham (4.67 $\pm$ 1.15 m/s) and AngII-treated (4.34 $\pm$ 1.48 m/s) aortas. However, the correlation coefficient ($\rho$) shows statistically significant difference (sham: 0.89 $\pm$ 0.03 versus the AngII-treated: 0.61 $\pm$ 0.15, $p < 0.001$).

Fig. 9(c) depicts the average value of the peak wall velocity in the proximal (P), middle (M) or aneurysmal (A), and distal (D) regions of the aorta, for both of the sham and AngII-treated aortas, respectively. As shown, the velocities of the sham aortas are relatively uniform in different regions. However, the velocities in the aneurysmal region of the AngII-treated aortas are lower than in the proximal region ($p < 0.005$). In addition, the velocities in the AngII-treated aortas are lower compared to the sham aortas ($p < 0.005$).

**E. Histology**

No aneurysms were found in the histology of the sham aortas. In the examples shown in Fig. 3(e), intact smooth muscle cells and elastin lamellae were observed in the sham aortas. Histology of the AngII-treated specimens revealed significant, but similar, changes in all mice that formed aneurysms. Many displayed intimal and/or medial ruptures with suprarenal sub-adventitial dissections starting to form. As evident in Fig. 5(e) and (f), H&E and EVG stains of an AngII-treated case demonstrate intact aortic segment and subadventitial hemorrhage, respectively. The aortas were undergoing extensive remodeling and inflammatory infiltration after AngII treatment. The ruptured specimen [Fig. 6(e)] revealed a break in the elastin lamellae with hemorrhage into the wall, formation of thrombus and contained on multiple cross-sections by the adventitia, and free hemorrhage around the aorta. This may be suggestive of a dissection, also occurring in the human case.

**IV. DISCUSSION**

The AAA is a complex multifactorial disease whose cause is currently unknown. The currently used criterion for AAA surgery is the maximum diameter of the abdominal aorta [2], [3]. However, small aneurysms are also known to rupture [7], [8] while some large aneurysms never do [9]. An imaging modality, which would be capable of assessing the potential of rupture or predicting the risk of rupture at an early stage, may efficiently reduce the enormous death toll associated with AAs.

The novel, noninvasive technique of PWI has been recently proposed to track and image the pulse wave during its propagation at high frame rates (8 kHz), along the abdominal aorta in mice in vivo [42], [43]. This method can provide regional variation of the wall velocity of the abdominal aorta at the time of pulse wave propagation, i.e., at the beginning of systole. Because disease typically causes regional vessel nonuniformity but not always changes the vessel wall geometry, it might be more useful to predict AAA disease at its early stage. In addition, because of the high resolution, we can gain new insights into the propagation pattern of the pulse wave along the aorta.

As illustrated in Figs. 2 and 3, in the normal and sham cases, the peak velocities remain uniform along the aorta. In the AngII-treated aortas (Figs. 4–6), the aneurysmal region undergoes the lowest velocity. In addition, the overall wall velocities of the AngII-treated aortas (Figs. 4–6) were lower than those of the normal and sham aortas (Figs. 2 and 3). Statistical results shown in Fig. 9(c) also confirmed these findings. The reduced pulse-wave induced wall velocity in the aneurysmal region reveals the stiffening of the aneurysmal aorta, which is consistent with the biochemical changes reported in Tham et al. [47], where increased collagen and decreased elastin content were reported in AngII-treated apolipoprotein E-deficient (apoE-KO) mice. The difference between the aneurysmal and the distal region are less significant ($p > 0.05$) [Fig. 9(c)], probably because the pulse wave gets affected by the aneurysmal region before it propagates to the distal region or because the distal region may also be aneurysmal.

As evident in Figs. 8 and 9, the correlation coefficients ($\rho$) of linear regression in the PWV measurement are reduced in the AngII-treated aortas. In other words, the propagation of the pulse wave is relatively uniform along the normal and sham aortas, but nonuniform along the AngII-treated aortas. The nonuniformity of the pulse-wave propagation is attributed to...
the inhomogeneity of the aortic wall properties after AngII infusion. With varying local stiffness, aortic diameter and wall thickness, various PWVs may be attributed to the same aorta according to (1). As a result, the propagation of the pulse wave in the region of interest would be nonuniform as confirmed by the PWI results shown in Figs. 4–6. In addition to the stiffness gradient and geometry change of the aortic wall along the longitudinal direction, the blood pressure change in the aneurysmal aorta may also affect the propagation of the pulse wave [48]. All these factors result in the complexity and nonuniformity of the pulse-wave propagation, which may require regional pulse wave information, especially in the presence of disease, diminishing thus the significance of a global PWV estimate. Ongoing work includes the modeling of the coupling of the aortic wall motion with the flow dynamics in order to investigate the role of stiffness, geometry and flow patterns in assessing the aortic wall mechanical properties and interpreting the PWI findings with a parametric analysis [48].

The velocities of the aortic wall were extracted through manual tracing of the wall on the B-mode images. When the pulse wave arrives, the aortic wall expands rapidly while the surrounding tissue is compressed and therefore undergoes similar motion, as shown in Figs. 2–6. Consequently, precise tracing of the aortic wall extent is not deemed a prerequisite for estimation of the spatio-temporal wall velocity and the regional PWV. In the normal case shown in Fig. 8(b), the PWV and correlation coefficient are $6.10 \pm 0.05$ m/s and $0.89 \pm 0.01$, respectively, from 10 different implementations of manual tracing. Therefore, the influence of human error (i.e., operator subjectivity) was confirmed to be comparatively small.

Preliminary statistical results [Fig. 9(a)] indicated that there was no significant difference in the PWV between the AngII-treated and sham aortas. This finding is not in agreement with the results of [49], in which increased aortic PWV was reported in AngII-treated mice. In that study, the aortic PWV was measured between the aortic arch to the abdominal aorta, across a distance of 40 mm [49]. This was not exactly the same as the suprarenal abdominal aortic PWV measured within 12 mm in our study. This discrepancy may also be due to variation among different mice in our experiments. The PWV could be affected by blood pressure, age, heart rate [49]–[51] and anesthesia [30]. All these factors could result in the variation of the PWV among mice. In order to obtain stable heart rates for ECG gating, the dose of the isoflurane varied between 1% and 2%, depending on the mouse. In the sham group, the heart rate (mean ± SD) was $444 \pm 63$ bpm. In the AngII-treated group, the heart rate was $406 \pm 83$ bpm. The physiologic variation was within the same range. This may explain the same variation in the PWV estimation between the sham and AngII-treated aortas. In addition to the PWV estimation, PWI maps the propagation of the pulse wave along the abdominal aorta, which can indicate a regional abnormality. This regional information of the propagation is thus deemed more complete than a single PWV value. For instance, the effects of age or diseases (e.g., hypertension) may affect the mechanical properties of the aortic wall in a global sense, while certain diseases (e.g., the AAA) may affect the regional mechanical properties. The longitudinal progress of AAA in the same mouse after AngII infusion is currently being investigated in order to reduce the variation among different mice as well as study the sensitivity of PWV estimation in detecting AAA and monitoring the mechanical property change during AAA development.

In the AAA model, the AngII was administered systemically and thus induced changes in the properties of the aortic wall globally along with generating the regional aneurysm. Although the exact mechanism of this model is still unclear, the aneurysm was consistently observed as a dilation of the aorta in the anterior suprarenal region, coincident with previous literature [44], [45], [52]. As shown in Figs. 2–6 and Fig. 9(c), the overall wall velocities were lower than those in the normal/sham cases, demonstrating that the entire aortic wall (even in the proximal region) had been affected by AngII. The effect of AngII was the most significant in the suprarenal region (i.e., at the level of the aneurysm formation), as indicated by the lowest wall velocity on the PWI images [Fig. 9(c)].

For a given imaging depth, there exists a tradeoff between the frame rate of the ultrasonic system and the FOV of the images acquired because of the finite speed of sound. The retrospective ECG gating overcomes this tradeoff [37]–[41]. With the high frame rate available in our system (8 kHz), the fundamental limit of the maximum PWV within an aortic length of 12 mm is 96 m/s, i.e., much higher than the physiological PWV (e.g., 2.2–8.5 m/s [30]).

The cost of ECG gating is that the images are combined from different cardiac cycles and not acquired in real time. In this study, a mechanical-sector scan with a single-element transducer and a line-by-line data acquisition were used to compose a full RF frame. The motion of the transducer from one position to another was fully controlled by the Vevo 770 system and could not be modified. Therefore, the approximate acquisition time for highest quality RF data was equal to 7 min for the FOV of 12 x 12 mm².

Recently, the ECG-gating technique was applied using a clinical ultrasound system in a clinical setting [53], [54]. Ultrasound imaging and RF data acquisition were controlled by the same program, with a phased array operating on a sector-by-sector basis. The full-view RF data of the human aorta was obtained from five to seven individual small sector frames. A total of 15–21 s, i.e., within a single human breath-holding period, was sufficient for a full scan with a maximum depth of 12 cm. By optimizing the transmit and receive schemes, the time interval could be further reduced; thus, making this system available for routine clinical applications. Preliminary PWI results in normal human [53], [54] and AAA aortas [55] have been reported.

V. CONCLUSION

The state-of-the-art technique of PWI was used to visualize the propagation of the pulse wave along the abdominal aortic wall of mice in vivo noninvasively, in normal, sham aortas and Angiotensin II (AngII) model of AAA. Different propagation patterns of the pulse wave were observed between normal/sham and aneurysmal aortas. The pulse wave propagated uniformly from the proximal to the distal side in normal and sham aortas, while it propagated nonuniformly in the AngII-treated aortas. In addition, the overall pulse-wave induced wall velocities of the AngII-treated aortas were significantly lower than those of
the normal/sham aortas. The velocities in the aneurysmal region were also lower than in the sham and AngII-treated aortas. The correlation coefficient ($r$) of the linear regression fit of the pulse-wave propagation and the peak wall velocities were shown capable of differentiating the AngII-treated aorta from the normal or sham aortas. PWI may be used to accurately depict the change in aortic wall properties as a result of AAA and potential rupture, before a change in the arterial diameter or other anatomical changes occurs or is detected. Finally, the same technique can be applied in the case of the other vascular diseases and conditions such as atherosclerosis, thrombus, hypertension, clot, and aging.

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