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In Vivo Vibroacoustography of Large Peripheral Arteries

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Abstract

Objective—Vibroacoustography allows imaging of objects on the basis of their acoustic signal emitted during low-frequency (kHz) vibrations produced by 2 intersecting ultrasound beams at slightly different frequencies. This study tested the feasibility of using vibroacoustography to distinguish between normal and calcified femoral arteries in a pig model.

Materials and Methods—Thirteen normal porcine femoral arteries, 7 with experimentally induced arterial calcifications, and 1 control artery injected with saline only were scanned *in vivo*. Images were obtained at 45 kHz using a 3 MHz confocal transducer. The acoustic emission signal was detected with a hydrophone placed on the animal's limb. Images were reconstructed on the basis of the amplitude of the acoustic emission signal. Vessel patency, vessel dimensions, and the extent of calcified plaques were confirmed *in vivo* by angiography and conventional ultrasound. Excised arteries were reexamined with vibroacoustography, X-ray radiography, and histology.

Results—*In vivo*, vibroacoustography produced high-resolution, speckle-free images with a high level of anatomic detail. Measurements of femoral artery diameter were similar by vibroacoustography and conventional ultrasound (mean difference $\pm$ SD, 0.1 ± 0.4 mm). Calcified plaque area measured by different methods was comparable (vibroacoustography, *in vivo*: 1.0 ± 0.9 cm²; vibroacoustography *in vitro*: 1.1 ± 0.6 cm²; X-ray radiography: 0.9 ± 0.6 cm²). The reproducibility of measurements was high. Sensitivity and specificity for detecting calcifications were 100% and 86%, respectively, and positive and negative predictive values were 77% and 100%, respectively.

Conclusions—Vibroacoustography provides accurate and reproducible measurements of femoral arteries and vascular calcifications in living animals.

Keywords

arterial calcifications; arterial stiffness; atherosclerosis; ultrasound; vibroacoustography

Arterial calcifications occur during the development of atherosclerosis and are predictors of cardiovascular risk in asymptomatic patients without prior coronary events.^{1–6} Although

coronary artery calcifications (CACs) do not specifically localize to vulnerable plaques, there is strong evidence of a direct relationship between overall CAC extent and cardiac event risk.¹ Testing for CACs can identify patients with subclinical atherosclerosis and allows targeted, aggressive risk factor modification to lower the incidence of cardiac events.⁴ Previous studies have documented the value of peripheral vascular calcifications for predicting coronary artery atherosclerosis and CACs,^{7–9} in addition to the traditional risk factors.¹⁰

Computed tomography (CT) is currently the most widely used noninvasive imaging modality for the quantitative assessment of total calcium burden on the cardiovascular system. However, there are concerns regarding the reproducibility of CT¹¹ and the X-ray exposure of sequential scanning, for example, to follow the effects of therapeutic interventions.^{12–15}

Vibroacoustography is a new ultrasound-based method to detect vascular calcifications.^{16–18} This technique uses acoustic radiation pressure produced by 2 intersecting ultrasound beams at slightly different frequencies to induce a vibration (kHz range) of the object from a distance. The image of the object is obtained on the basis of the vibration-induced acoustic emission signal. This signal depends on the stiffness as well as the acoustic properties of the object.^{17, 19}

Vibroacoustography can easily detect calcified deposits in excised tissue specimens scanned in a water bath. The resulting images faithfully resemble X-ray images of specimens.^{16–22} However, in vivo, numerous factors could distort the ultrasound and acoustic emission signals.

To our knowledge, this is the first in vivo study to test the ability of vibroacoustography to image normal femoral arteries and to detect calcified plaques in femoral arteries of anesthetized swine, an animal model that allows rapid and reproducible plaque formation.²³

MATERIALS AND METHODS

We used a swine model of artificially induced arterial calcifications.²³ The advantage of this model is that calcified lesions can be easily, quickly, and reliably induced within days. We scanned porcine femoral arteries because they are readily accessible using the available setup for vibroacoustography. All experiments conformed to the “Position of the American Heart Association on Research Animal Use” and to the Institutional Animal Care and Use Committee guidelines for the care and use of laboratory animals at our institution.

Eleven farm pigs weighing 30 to 40 kg were used in this study. Animals were anesthetized (induction: ketamine, 20 mg/kg, and xylazine, 2 mg/kg, intramuscularly [IM]; maintenance: intravenous [IV] infusion of ketamine, 11.52 mg · kg⁻¹ · h⁻¹, and xylazine 1.5 mg · kg⁻¹ · h⁻¹, plus supplemental tiletamine zolazepam bolus, 1.4 mg/kg, as needed), intubated, and allowed to breathe spontaneously. A 7F or 8F guiding catheter was inserted into the femoral artery, via the left or right carotid artery, for angiography and injection of echocardiographic contrast agent (Optison, Mallinkrodt Inc., St. Louis, MO) during scanning. Animals received heparin (1000 IU/h) to prevent thrombus formation and ketorolac tromethamine (60 mg IM) and methylprednisolone (40 mg IV) to prevent pulmonary macrophage reaction to Optison. Vessel patency was confirmed by angiography and B-mode ultrasound.

Induction of Arterial Calcifications

After baseline imaging, the animal was brought back to the operating room. The left or right femoral artery was dissected free, and calcium hydroxyapatite solution (dissolved in 6N HCl) was injected into the adventitial layer. This animal model was introduced by Ashai et al.²³ Within days after injection, the calcium solution infiltrates the arterial wall where it precipitates

and develops a hard plaque with necrosis, inflammation, and stenosis resembling humanoid plaque.

In our pigs, we injected a smaller dose of hydroxyapatite solution in a total of 7 arteries (0.1 mL: 2 arteries; 0.03 mL: 4 arteries; and 0.01 mL: 1 artery) to obtain less extensive plaques without severe stenosis.

The control animal received an injection of 0.03 mL of saline intra-adventitially to test the effect of the surgical procedure on the vibroacoustographic appearance of femoral arteries.

The wound was closed, and the animals were allowed to recover from anesthesia. Infection was prevented with antibiotics. Vibroacoustographic scans were repeated after 1 to 2 weeks. After euthanasia (pentobarbital overdose), femoral arteries were harvested for in vitro studies.

In Vivo Vibroacoustography

Vibroacoustographic imaging was performed in 13 normal femoral arteries. In the 7 arteries injected with calcium hydroxyapatite, scans were performed 7 or 14 days after the injection. The reproducibility of vibroacoustographic imaging in vivo was tested in 6 normal femoral arteries that were scanned twice, at a 1-week interval.

After surgical instrumentation, vibroacoustography was performed in a dedicated, vibration-isolated room. The animal was lying in a supine position on an air suspension table (Fig. 1A). A water bath made of a thin plastic sheet was glued (contact cement) on the animal's groin and filled with warm, degassed water. The bottom of the bath had a 10-cm circular opening to allow unimpeded access of the ultrasound beams to the animal's limb. A dual-beam confocal transducer (45-mm diameter) mounted in the bath was used to send 100-microsecond tone bursts to produce 45-kHz vibrations resulting from the intersection of 2 ultrasound beams at slightly different frequencies (2.980 and 3.025 MHz). The ultrasound signals were produced by 2 signal generators (model 33120A, Agilent Technologies, Palo Alto, CA). The focal point of this confocal transducer is fixed at 7 cm from the aperture. The focal region is approximately 1 mm wide and 10 mm long. The transducer was positioned according to the depth and location of the femoral artery. The average depth of the femoral artery (midlevel) was 1.2 ± 0.1 cm under the skin in normal pigs and 1.8 ± 0.5 cm at 1 to 2 weeks after surgery.

The acoustic emission signal was detected by a hydrophone (model 6050C, ITC, Santa Barbara, CA) placed outside the bath directly on the animal's limb. Ultrasound gel was used to enhance the acoustic coupling. The signal was digitized at 100 kHz over a 100-microsecond period, producing 10 time-slice images spaced at 10-microsecond intervals. The recorded signal was band-pass filtered (model SR650, Stanford Research Systems, Sunnyvale, CA), and amplitude-based images were reconstructed in the computer. Raster scans of 5×5 cm were obtained in a coronal plane passing through the artery (Fig. 1A). The spatial sampling interval was 0.25 or 0.50 mm (100 or 200 points/scan line). One image scan took 3 or 6 minutes, depending on the resolution used. Images were acquired at different depths (up to 20 images per study). The image that best visualized the artery and the plaque was selected for quantitative analysis. A spatial low-frequency band-pass filter (Photoshop, Gaussian filter, size 50, Adobe Systems, San Jose, CA) was applied to all images to compensate for the stronger acoustic emission signal for points closer to the hydrophone position (Fig. 1A).

In our settings, the pulse peak intensity measured in water was 130 W/cm^2 (for <1% duty cycle), and the spatial peak temporal average intensity was $<1.3 \text{ W/cm}^2$. In vivo, this intensity would be $<0.775 \text{ W/cm}^2$, considering the average frequency-dependent attenuation of muscle and fat tissue (0.5 dB/cm/MHz), the frequency of the transducer (3 MHz), the depth of porcine femoral arteries (1–2 cm), and the speed of vibroacoustographic scanning (1 scan every 3–6

minutes). Therefore, the intensity is comparable to or lower than the limit recommended by the US Food and Drug Administration (FDA). The mechanical index was <1 .

Conventional Ultrasound

B-mode and M-mode ultrasound were performed using a 10-MHz transducer and a Vivid 7 scanner (GE Healthcare, Milwaukee, WI) before vibroacoustographic scanning. Femoral artery diameter was measured at 3 locations within a 5-cm segment. Plaque size was also measured from B-mode images.

In Vitro Vibroacoustography

Excised arteries (6–8 cm in length) comprising the plaque and adjacent normal segments were immersed in normal saline, and vibroacoustographic scanning was performed the following day. Arteries were mounted on a frame (glued to a latex membrane or attached by strings) and scanned in a water tank filled with degassed water, using a previously described setup.^{17–22} Briefly, the confocal transducer was used to send a 10-kHz amplitude-modulated continuous-wave signal. The hydrophone was submerged in the water tank. The frame containing the artery was placed in the focal plane of the transducer, and scanning was performed in the plane of the artery.

High-Resolution Radiography

Excised arteries were scanned with a high-resolution specimen radiography machine (Faxitron model M50, Wheeling, IL) to document the presence and location of the calcifications and to measure plaque extent.

Histology

Artery specimens were immersed in 10% formalin and sent for histologic analysis (injected: 2; control: 1). Arteries were embedded in paraffin, and 5- μ m cross-sections were cut at 3-mm intervals (15–20 sections per artery). Slides were stained with hematoxylin and eosin and Verhoeff–van Gieson. A stenosis grade was assigned to each artery according to the most severely stenosed cross-section (mild: 0%–25%; mild–moderate: 26%–50%; moderate: 51%–75%; and severe: $>75\%$). Plaque composition was assessed semiquantitatively and included fibrosis, calcifications, necrosis, and fibrointimal proliferation. These components were expressed as a percentage of the total plaque area for a sum of 100%.

Quantitative Analysis

All in vivo vibroacoustographic images were interpreted by 2 investigators, one of whom was unaware of animal identifiers (blinded analysis). Calcified plaques were manually delineated, and plaque length and area were measured in both in vitro and in vivo vibroacoustographic images as well as on radiographic images. Measurements of the femoral artery diameter by vibroacoustography were compared with those obtained using conventional ultrasound. The diameter of the guiding catheter measured from 5 in vivo vibroacoustographic images was compared with the manufacturer-provided size. Differences between measurements are reported as absolute values (in mm) and in percentage relative to the conventional ultrasound measurement.

Influence of Pulse Pressure and Vessel Distention

To test whether systolic–diastolic artery distentions might have influenced the vibroacoustographic appearance of femoral arteries, we performed an in vitro experiment to simulate and control for in vivo conditions. We used a latex rubber tube (6 mm in diameter, 1.5 mm in wall thickness) with elastic properties comparable to those of porcine femoral

arteries. The tube was embedded in gelatin. A diaphragm pump was used to create pressure oscillations in the tube (Fig. 1B). The pulse pressure was chosen at a level to produce tube distention of a similar magnitude (~0.21 mm) as that measured in femoral arteries of pigs in vivo. Pressure was measured using a 3F Millar catheter and recorded simultaneously with the acoustic emission signals. The pressure oscillations were created using the diaphragm pump controlled by an electronic signal (Agilent Technologies) adjusted to simulate a pressure waveform similar to the femoral artery pressure waveform. Vibroacoustographic scans (3 × 5 cm) were performed with the tube at constant pressures of 60, 80, 120, 140, and 160 mm Hg (using a water column) and at 60/120 and 60/160 mm Hg (minimum/maximum) dynamic pressures (at 1 Hz). Pressure-induced variations of the acoustic emission signal were also measured at a single point location in the tube wall (closest to the transducer), during a 5-second interval, at a pulse pressure of 100 mm Hg (60–160 mm Hg).

Statistical Analysis

Values are presented as mean ± SD. To determine the agreement between measurement methods, the intraclass correlation coefficient (ICC) was calculated.²⁴ The ICC compares the variability of measurements between the different reading methods on the same subject to the total variability across all methods and all subjects. The ICC ranges between 0 and 1 and values closer to 1 signify within-subject variability smaller than between-subject variability. Variability estimates were obtained by fitting a fully crossed 2-way ANOVA model with subjects as random effects and methods as fixed effects. The agreement between measurements is also presented using Bland–Altman graphs.²⁵ Statistical significance was inferred for $P < 0.05$.

RESULTS

Thirteen normal femoral arteries were scanned in vivo. Seven femoral arteries were injected with calcium hydroxyapatite solution to induce calcified plaque and 1 control artery was injected with normal saline. Angiography and B-mode ultrasound confirmed that all arteries were widely patent and did not show stenosis or intraluminal thrombus.

Gross Pathology and Histologic Findings

On gross pathology, calcified plaques extended 1–3 cm along the artery. Histologic analysis confirmed calcium deposit in the subintima, media, and adventitia, involving 30% to 80% of the arterial circumference (Fig. 2). All stenoses were mild. Plaques showed predominantly calcium (5%–80% of the plaque's cross-sectional area) and fibrosis (10%–80%). Mild necrosis of the adventitia and media, with disruption of the external elastic lamina, was present in 10% to 20% of the arterial circumference. Fibrointimal hyperplasia was observed in 30% to 70% of the arterial circumference. In the control animal, saline injection did not produce notable histologic abnormalities in the injected segment (Fig. 2).

Vibroacoustography of Normal Arteries In Vivo

Figure 3 shows the in vivo vibroacoustographic images of normal femoral arteries in living swine. Vibroacoustography produced high-resolution, high-contrast, and speckle-free images. Considerable anatomic detail could be observed, including the course and boundaries of the femoral artery and its diameter and branching anatomy within the imaging plane. Artery walls appeared straight, relatively thin, and smooth. The brightness of the vessel walls varied regionally along the length of the artery. The lumen appeared dark or dense and had an intensity similar to or lower than the artery walls and surrounding muscles. Injection of air-filled microbubbles produced a signal much stronger than the surrounding tissue and confirmed the course and boundaries of the artery (Fig. 3D).

Figure 4A shows vibroacoustographic images of the control animal before and 7 days after intra-adventitial injection of saline. These images showed that the surgical procedure and saline injection did not disturb the normal vibroacoustographic appearance of the artery. Histology confirmed this finding (Fig. 2). Vibroacoustographic scans obtained 1 week apart in the same animals demonstrated the good reproducibility of the technique several days after the initial scan (Fig. 4B).

Vibroacoustography of Calcified Arterial Plaques In Vivo

Figure 5 and Figure 6 show examples of in vivo vibroacoustographic images several days after injection of calcium hydroxyapatite. Calcified plaques appeared as irregularities along a thickened artery. Large plaques appeared as dark defects. On the basis of the branching anatomy, the location of these plaques matched the location and extent of calcifications seen on ex vivo vibroacoustographic scans and X-ray radiographs of the excised specimens. The normal proximal and distal segments (1–2 cm) served as reference of a normal artery in each animal.

Figure 7 illustrates examples of in vivo vibroacoustographic scans acquired at different tissue depths, supporting the potential of vibroacoustography to image the atherosclerotic plaque in its 3D configuration.

Reproducibility of Vessel Dimensions and Plaque Extent

We found very good agreement between vibroacoustography and all other methods for measurement of vessel diameter and plaque extent (Table 1). Figure 8 shows Bland–Altman graphs for agreement between methods. The mean diameter difference by vibroacoustography and conventional ultrasound was 0.1 ± 0.4 mm (relative difference: $5\% \pm 9\%$; $P = \text{NS}$). The interobserver variability was 0.2 ± 0.7 mm ($4\% \pm 13\%$), and the intraobserver variability was 0.2 ± 0.4 mm ($5\% \pm 10\%$). Measurements of the catheter diameter had a slightly smaller variability (0.1 ± 0.2 mm; $2\% \pm 7\%$).

The intraindividual variability in measurements of artery diameter (day 1 versus day 7) was also very good (Fig. 8). The mean difference in measurements was 0.1 ± 0.2 mm ($2\% \pm 5\%$).

Plaque size measurements by different methods were also similar, and differences were not significant (Table 1). On blinded analysis, the image reader correctly identified the presence of calcified plaques on all in vivo images of arteries with ex vivo confirmed plaques. Two (15%) of the 13 normal arteries were incorrectly classified as calcified. The sensitivity and specificity for detecting calcifications were 100% and 86%, respectively. The positive and negative predictive values were 77% and 100%, respectively.

Influence of Pulse Pressure and Arterial Distention

Figure 9A shows vibroacoustographic images of the latex tube scanned during pressure oscillations, which simulate in vivo artery pulsations and random scanning during the cardiac cycle, and during static pressures, which simulate in vivo electrocardiographically gated image acquisitions. We found no visible effect of vessel distention during static or pulsed pressure on the tube's appearance in vibroacoustographic images. There was only a minor (3%) decrease in the amplitude of the acoustic emission signal with pressure (Fig. 9B, C).

DISCUSSION

In this study, we demonstrate that vibroacoustographic imaging of normal and calcified femoral arteries is feasible in vivo. The high-resolution vibroacoustographic images are speckle-free and provide detailed information on the local morphology of femoral arteries, which allow

accurate measurements of anatomic structures with high reproducibility and sensitivity. In our settings, the estimated spatial peak time average intensity was within the FDA-recommended limit, which suggests that vibroacoustography is suitable for serial imaging.

Vibroacoustography: Technical Details

Vibroacoustography allows visualization of objects on the basis of their acoustic signal emitted during low-frequency (kHz) vibrations. The acoustic emission depends on the stiffness and ultrasound characteristics (scattering and absorption) of the object and the surrounding medium. Highly localized radiation force is produced at the intersection of 2 ultrasound beams of slightly different frequencies to “remotely” tap the tissue (at the difference frequency, Δf) and detect hard inclusions in the tissue.^{16,17} This method has better displacement sensitivity (subnanometers) than other elastography methods.^{17,19,26} The spatial resolution depends on the beam width at the focal plane and on the transmitted ultrasonic frequencies. Because the acoustic emission response is in the kHz range, signal attenuation is low and ambient noise can be filtered out. Several potential clinical applications have been explored in vitro, including monitoring of temperature-related changes of tissue elasticity²⁷ and flow measurements.²⁸ Following a study in living animals,²⁹ one group also described the potential of vibroacoustography for the detection of breast mass lesions containing calcifications in breast cancer patients.³⁰ In that study, imaging was performed using a new vibroacoustographic system dedicated to breast imaging. However, no quantitative results were reported.

The contrast in vibroacoustographic images depends not only on stiffness but also on the ultrasound characteristics (scattering and absorption) of the object and the surrounding medium. Thus, images contain unique information compared with those generated by conventional ultrasound. Generally, hard inclusions such as microcalcifications produce a high spatial variation in the energy density of the acoustic field and, therefore, a strong acoustic emission signal. However, objects with large mass (eg, large calcified plaques) are hard to move and, thus, may emit a weak acoustic emission signal. The exact relationship between the generated acoustic signal and object hardness is complex and not completely understood. Nevertheless, these images provide useful and unique information, with a level of detail superior to conventional ultrasound at similar transmitted frequencies (due to the absence of speckles).

Other novel methods to assess arterial elasticity include intravascular ultrasound elastography, which may be able to characterize plaque composition by mapping intramural deformation.³¹ Another method is acoustic radiation force impulse imaging, which applies brief, high-energy, focused acoustic pulses to generate a mechanical “push” in the tissue and measures the response of the tissue to the applied force.³²

In Vivo Vibroacoustography

In this study, we demonstrate that, using vibroacoustography, we can obtain detailed information on local morphology of normal and calcified femoral arteries. In our study, vibroacoustography had high sensitivity but slightly reduced specificity for the detection of vascular calcifications. Plaque extent was similar in both in vivo and in vitro vibroacoustographic images, and there were no notable differences when the images were compared with those generated by other modalities (ie, in vivo ultrasound and ex vivo radiography of arterial specimens).

An important advantage of vibroacoustography is the absence of speckles, which are characteristic with the conventional ultrasound. Speckles reduce image quality and decrease the level of detail displayed and the diagnostic accuracy of conventional ultrasound. Although we did not attempt detailed, noninvasive plaque characterization, our data suggest that

vibroacoustography may be suitable for this purpose, given its ability to image plaques 3-dimensionally and its theoretical ability to detect regional variations in stiffness. This ability is important because plaque composition and spatial distribution of its components may be more important than the absolute quantity of calcium in the plaque.^{33,34} In particular, a scattered and spotty calcification pattern in individual fibrofatty plaques is typical for culprit lesions in acute myocardial infarction and may identify plaques prone to rupture.³⁵

In our study, interobserver and intraobserver variabilities for morphologic measurements by vibroacoustography were at least as good as those derived by other imaging methods. Our data suggest high sensitivity but slightly reduced specificity of vibroacoustography for identification of calcium deposits. In normal femoral arteries, there was some variability in signal intensity along the artery walls, which was likely responsible for the misclassification of 2 of 13 arteries as calcified. Several explanations are possible: variations of the signal path,^{16–19} influence of other structures adjacent to the artery, regional inhomogeneities in the acoustical and mechanical properties of arteries that cannot be appreciated with other imaging techniques, and possibly other factors. We tested whether pressure variation could be a major factor because this effect would have appeared randomly in our *in vivo* scans. Our *in vitro* tests showed that vessel pulsations and blood pressure level did not have a major impact on the vibroacoustographic appearance of femoral arteries in our pigs. These results are expected because the spatial resolution in our vibroacoustographic scans was smaller than the magnitude of femoral artery systolic–diastolic distention.

Future clinical studies on human subjects and using improved equipment will substantiate the value and accuracy of vibroacoustography. Technologic advances will improve the speed of data acquisition and the spatial resolution,³⁶ allowing imaging of more complex pathology. New transducer designs (phased arrays) are expected to increase the image spatial resolution (ie, depth resolution, 7 mm versus 10 mm) and frame rate (up to 1 Hz for a 100 × 100-pixel image at a 50-kHz difference frequency).³⁶ Current developments indicate that vibroacoustographic scanning may be possible using a modified commercial scanner (eg, for a 7-MHz linear transducer, spatial resolution in vibroacoustography would be about 0.4 mm in azimuth and 1.5 mm in elevation). As a possible clinical use, vibroacoustography could guide quantitative measurements of arterial stiffness using other complementary techniques at specific locations,^{37–39} where the existence of plaques would be suspected on the basis of vibroacoustographic and conventional ultrasound surveillance.

An interesting finding in this study was the decrease in the acoustic emission signal with pressure, although this decrease was of small magnitude (3%). One explanation could be the increase in tube stiffness (as a structure) with pressure, reducing the vibrations induced by acoustic radiation. Because latex is an elastic material over the range of pressure tested (elastic modulus should not vary with pressure), changes in material properties cannot explain our experimental finding. Nevertheless, the relationship between stiffness (or pressure) and acoustic emission is complex and requires further studies to understand this relation.

The presence of air-filled microbubbles in the vessel lumen increased the acoustic emission signal. This is an expected finding. Bubbles are highly nonlinear and the 2 ultrasound frequencies mix (multiply) efficiently in the bubbles, thus making an especially loud difference frequency signal. Earlier *in vitro* studies with contrast microbubbles showed that the acoustic emission signal is proportional to the bubble concentration.²⁸ Future studies will address this additional potential application of this technique.

Limitations

The animal model used does not reproduce biologic plaque development, which typically includes smooth muscle cell proliferation and lipid accumulation,^{40–42} but it is an excellent

model of rapid arterial calcification. We did use calcium hydroxyapatite, which is the main component of calcified atherosclerotic plaque in patients. The number of animals is small and the accuracy of vibroacoustographic measurements may vary in larger studies. Nevertheless, to our knowledge, this was the first in vivo test, and future studies will establish the value of this new technique.

Electrocardiographically triggered imaging would be desirable, but it was not technically feasible at this point and it would have increased the acquisition time by a factor of 50 or more. However, an electrocardiographically gated approach will be needed for more elastic arteries with large deformations. Further developments will allow shorter scanning times and triggered imaging.³⁶

We used different frequencies for in vitro and in vivo imaging by choosing settings that are optimal for each condition. Higher difference frequencies (Δf) give a stronger acoustic emission (needed in vivo), but lower frequencies allow the best visualization of plaque compared with the gold standard, X-ray images.^{17–22} We used a relatively low-frequency transducer (3 MHz) as a trade-off between the magnitude of the acoustic emission obtained and the image resolution. However, higher-frequency transducers may be used.

Summary

Vibroacoustography provides speckle-free images of local anatomy and can easily detect arterial calcifications in the peripheral arteries of large animals. In our experiments, the spatial peak time average intensity was estimated to be within the FDA-recommended limit, which suggests that vibroacoustography is suitable for serial imaging. Further studies are warranted to evaluate the potential of this technique for identifying noncalcified atherosclerotic lesions and to differentiate between stable and unstable plaques on the basis of the acoustic emission signal.

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FIGURE 1.

A, Diagram of the in vivo experimental setup for vibroacoustographic imaging. B, Diagram of the in vitro experimental setup to test the influence of vessel pulsations.

Calcium hydroxyapatite

Control (saline)

FIGURE 2.

Hematoxylin-eosin (left) and Verhoeff–van Gieson (right) staining of femoral arteries injected with calcium hydroxyapatite solution or saline. Top images: Injection of calcium hydroxyapatite solution produced mild to severe structural alterations in all 3 artery layers. Moderate and severe intimal hyperplasia, medial and adventitial necrosis, and extensive calcium deposition were found in the plaque. *Arrowheads* outline the calcification forming a plaque in the media and the more ill-defined particles forming a nodule in the adventitia. Verhoeff–van Gieson staining outlines the different layers and allows localization of calcifications. Bottom images: No abnormal changes were found after saline injection. (Magnification: X40; scale bar: 1 mm.)

FIGURE 3.

In vivo vibroacoustographic images of left (A) and right (B–D) normal femoral arteries from 3 pigs (C and D images were taken from the same animal). Images are reconstructed on the basis of the amplitude of the acoustic emission signal at 45-kHz vibrations. Scans were obtained in a coronal plane passing through the artery (see text for details). *L* indicates the lumen. The D image shows vibroacoustographic images during injection of air-filled microbubbles. Arrows indicate the tip of the catheter. (Scale bar: 1 cm.)

FIGURE 4.

A, Vibroacoustographic images of the left femoral artery from the control animal before (left) and 7 days after (right) intra-adventitial injection of saline. B, Examples of vibroacoustographic images from a normal femoral artery obtained at a 1-week interval, demonstrating the good reproducibility of this imaging method between serial scans. (Scale bar: 1 cm.)

FIGURE 5.

Examples of images obtained from 1 animal with a calcified plaque. A, In vivo vibroacoustographic imaging was performed at 7 days after calcium hydroxyapatite injection. B, Contrast angiography at 14 days showed no notable stenosis at the location of injection (at the tip of the instrument). C, Photograph of femoral artery in situ showing the calcified plaque. D, E, Vibroacoustographic and X-ray images of the excised specimen.

FIGURE 6.

Data obtained from another animal with a calcified arterial plaque. In vivo vibroacoustography was performed at baseline (A), 7 days (B), and 14 days (C) after calcium hydroxyapatite injection. B-mode ultrasound image at 14 days after injection (D). Photographic (E), vibroacoustographic (F), and X-ray (G) images of excised artery containing the plaque are shown for comparison. (Scale bar: 1 cm.)

FIGURE 7.

Vibroacoustographic images of femoral artery shown in Figure 6C, at 14 days after calcium hydroxyapatite injection. Images were obtained at 4 depth levels (in 2-mm increments) and show the variation in plaque area (*arrows*) with depth. (Scale bar: 1 cm.)

FIGURE 8.

Bland–Altman graphs showing agreement between vibroacoustography (VA), ultrasound (US), and X-ray radiography. The differences between measurements are plotted against their means. Horizontal lines represent limits of agreement.

FIGURE 9.

A, Vibroacoustographic images of the latex tube at 60 to 160 mm Hg pulsed pressure and at constant 60 and 160 mm Hg pressures. There is no visible effect of pressure and tube pulsations on these images. Tube distention was 0.2 mm between 60 to 160 mm Hg. The bright spot represents an acrylic bead. *Arrow* indicates the tip of the pressure catheter. (Scale bar: 1 cm.) B, Variations in the acoustic emission (AE) signal at one location in the tube wall during 100 mm Hg pressure oscillations. There was a slight (~ 12 mV) decrease in the AE with pressure. C, The temporal relation between the mean AE (*black thick line*) and pressure (*blue line*) is shown on the right. The black dashed lines represent mean AE $\pm$ SD. The scatter plot on the bottom shows the correlation between the magnitudes of the 2 signals.

TABLE 1

Femoral Artery and Plaque Extent

	In Vivo		In Vitro	
	Vibroacoustography	Ultrasound	Vibroacoustography	X-ray
Artery diameter (mm)	4.7 ± 0.8	4.6 ± 0.7	—	—
Plaque length (cm)	2.0 ± 1.0	1.8 ± 0.9	2.1 ± 0.6	1.9 ± 0.9
Plaque area (cm ²)	1.0 ± 0.9	—	1.1 ± 0.6	0.9 ± 0.6
				ICC (95% CI)
				0.97 (0.82–1.00)
				0.99 (0.93–1.00)

ICC indicates interclass correlation coefficient; CI, confidence interval.