Published in final edited form as:

Ultrasound Med Biol. 2005 October ; 31(10): 1351–1359.

Correspondence of Ultrasound Elasticity Imaging to Direct Mechanical Measurement in Aging DVT in Rats

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Abstract

Previous ultrasound elasticity imaging experiments supported a generally accepted concept that the hardness of deep venous thrombi increases with thrombus aging. Results also showed that this non-invasive imaging technique can accurately predict thrombus age through strain estimates in a well controlled animal study. In the present study, as an alternative means to characterize elastic properties of thrombi, we employed a direct mechanical measurement system to estimate Young's modulus of *ex vivo* thrombi. Unlike conventional indentation tests, the device utilizes a specific compression geometry for cylindrical tissue specimens. We also proposed an approximation scheme to retrieve Young's modulus from force-displacement measurements made using the device. Finite element simulations and calibrations on tissue-mimicking phantoms validated the system. Then, using two groups of rats with surgically induced thrombi, we further investigated the correlation between Young's modulus measured *ex vivo* and elasticity images reconstructed *in vivo*. This comparison was accomplished by converting the intra-thrombus strains measured in the *in vivo* studies into Young's modulus estimates using a model based approach. Good agreement between time-dependent Young's modulus estimates observed *in vivo* and direct measurements of Young's modulus using the mechanical device helps to confirm the ability of elasticity imaging to age DVT for efficient treatment.

Keywords

Deep venous thrombosis; Mechanical measurement; Ultrasound elasticity imaging; Elasticity reconstruction; Young's modulus

INTRODUCTION

Deep venous thrombosis (DVT) and its associated complication of pulmonary embolism (PE) are significant medical problems. It has been estimated that there are about 2 million new cases of DVT per year in the United States. Of these, approximately 600,000 patients will develop PEs resulting in about 60,000 deaths (Hirsh and Hoak 1996). This high mortality rate makes it imperative to appropriately treat a DVT once it has been identified. Treatment depends on the maturity of the thrombus, with more acute thrombi requiring heparin therapy with its concomitant increased bleeding risk. The standard maturity criterion is thrombus age with older

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thrombi being considered more mature. It is well known that thrombus age is closely related to thrombi hardening (Lewis 1998; Browse et al. 1999). Based on this relationship, it has been shown that ultrasound elasticity imaging does a remarkably accurate job of estimating the age of thrombi in rats (Emelianov et al. 2002; Xie et al. 2004). Using a 2D phase-sensitive speckle tracking technique to monitor deformations of inferior vena cava (IVC) thrombi, we have been able to predict the ages of surgically induced thrombi in rat IVC to within ± 0.8 days for thrombi that are ten days old or less.

In our previous studies (Emelianov et al. 2002; Xie et al. 2004), hardness estimates were based on strain measurements normalized to the average surface applied strain, which was calculated as the ratio of the accumulated spine displacement to the baseline axial depth of the spine anterior surface. Thrombus maturity was modeled through the normalized strain as an exponential decay function of thrombus age (Xie et al. 2004). Such normalized strain estimates rely on the assumption that the tissue overlying the spine is generally similar from rat to rat. These estimates are, of course, influenced by the well-known strain intensification effects that occur at boundaries of tissues of different Young's modulus during deformations (Ponnekanti et al. 1994; Kallel et al. 1996). Strain distributions are also influenced by boundary conditions and assumptions regarding homogeneity of the materials surrounding thrombi (Skovoroda et al. 1995; Kallel et al. 1996; Doyley et al. 2000). Thus, strain measurements provide only approximate measures of the underlying Young's modulus distribution in tissue. It is, therefore, imperative that strain estimates of thrombus hardness be validated by direct measurements of Young's moduli in thrombi. It will then be possible to determine the accuracy of the estimates, at least in a highly controlled situation such as the rat IVC. If these estimates are not reliable, it is highly unlikely that estimates of thrombus hardness, represented by Young's modulus, in a more complex environment such as in human femoral and calf veins will carry much predictability. Only in this way will investigators be confident that strain estimates in DVT actually reflect the true Young's modulus, and the maturity, of underlying thrombi.

In the literature, a number of mechanical measurement systems have been developed to characterize the elastic properties of soft tissue. For tissue specimens that can be excised into large homogeneous pieces, for example, liver and muscle, the traditional approach is uniaxial compression (Chen et al. 1996). For small tissue samples (breast and prostate), a majority of existing systems are indentation apparatus (Erkamp et al. 1998; Krouskop et al. 1998; Samani et al. 2003). Their general procedure is to indent the tissue sample in either constrained or unconstrained lateral boundary conditions, record the indenter displacement, simultaneously measure the resulting deformation force, and then convert the slope of force-displacement to Young's modulus via a conversion factor. Samani et al. (2003) proved that in the linear theory of elasticity, the conversion factor is only related to boundary conditions and deformation geometry. The factor can be determined by theoretical derivation (Hayes et al. 1972), finite element simulation (Erkamp et al. 1998; Samani et al. 2003) or calibration with phantom samples of known Young's modulus (Erkamp et al. 1998; Samani et al. 2003). The mechanical device presented in (Krouskop et al. 1998) is capable of testing tissue samples (breast tissue) prepared in a thin slab. Canine kidney samples were characterized using the system developed by Erkamp et al (1998). Samples were cut into cylinders and constrained on the side and bottom in a mold via gelatin. A circular piston indents the center of the sample. Samani et al. adapted the basic concept of (Erkamp et al 1998) in designing their system. However, tissue samples (breast tissue) were measured in a block shape.

Thrombi are the focus of direct mechanical measurement in this paper. In our animal study, thrombus is formed in rat IVCs by ligation induced stasis. The cylindrical shape of the vessel constrains the thrombus geometry, so thrombi can be approximately modeled as a cylinder, especially when they are acute and occlusive. Housed by the IVC, the diameter of the thrombus cross-section is usually no larger than 4 mm and the maximum thrombus length is around 1.5–

2 cm. Thus, in cross-section, thrombi are much smaller than tissue samples best suited to the aforementioned systems. If we simply adopt one of the above systems and scale down the size accordingly, the indenter might puncture and damage thrombi during compression. This points to the need for a device specifically designed to measure the Young's modulus of thrombi. During *in vivo* ultrasound elasticity imaging experiments, the thrombus-containing IVC is deformed in the transverse plane (i.e., the cross-section plane perpendicular to the long axis of a cylinder) by a force exerted through an ultrasound transducer. The new device should therefore be able to measure thrombi in a deformation geometry that closely replicates the *in vivo* measurements.

In this study, we developed a measurement system that uses a rectangular piston to deform *ex vivo* thrombus along a transverse plane similar to the deformations performed using a transducer *in vivo*. Then we performed measurements *in vivo* and *ex vivo* on the same thrombi in the same orientation in a group of rat IVC thrombi, and compared these results to another set of rat IVC thrombi performed without imaging as a negative control. This allows us to determine if the act of deforming IVC thrombi *in vivo* actually changes the Young's moduli. Finally, we compare the *ex vivo* direct Young's modulus measurements with a group of rats for which the Young's moduli have been reconstructed from strain estimates of IVC thrombi using a model based approach. Using this technique, we are able to compare the true Young's modulus based on mechanical measurements with predicted *in vivo* thrombus hardness based on elasticity imaging.

METHODS

Mechanical Measurement Device

A desktop electronic device named MicroElastometer (Artann Laboratories, Lamberville, NJ) was developed for accurate measurement of elastic properties of thrombi. Similar to the aforementioned devices, the MicroElastometer measures the displacement versus force while a test sample is subject to compression. Based on the strain-stress relationship, Young's modulus of the test sample can be further estimated. The device is shown in Fig. 1a. A zoomed-in version is shown in Fig. 1b where a cylindrical rubber phantom is placed on the object plate to mimic how a thrombus modulus would be measured. A pressing stamp with a rectangular cross-section will move down to compress the cylinder in its transverse plane.

The compression force is measured by a strain gage (Acculab VI-200, Acculab, Edgewood, NY) mounted under the object plate. The measurable force range is up to 2 N with a precision of 0.1 mN. The position of the compression stamp is controlled by a hybrid linear actuator (HSI 35N67-12-405, Haydon Switch & Instrument Inc., Waterbury, CT). The minimum travel distance per step is 3 μ m. The MicroElastometer uses a bi-directional RS-232 interface to communicate with the user's computer through serial and parallel ports. The user interface of the MicroElastometer system software allows the user to calibrate the device, set up the measurement protocol, and measure the sample. The user can specify the sample geometry and choose the number of steps that the stamp travels between two consecutive measurement points. Besides cylindrical test samples with a circular cross-section, the device is also capable of measuring samples prepared in two more shapes: layers with a circular or rectangular cross-section larger than that of the stamp, and strips with width narrower than the length of the stamp. However, we will only focus on cylindrical samples. During measurement, linear actuator position, force reading as well as the run-time force-strain curve are updated in real time in the user interface. When a measurement is completed, the Young's modulus will be estimated and immediately displayed.

The pressing stamp has a cross-section with dimensions of 8.88 mm by 3.98 mm. As part of the measurement protocol, cylindrical samples are required to be positioned perpendicularly

to the long side of the stamp, unconfined in the lateral direction, as demonstrated in Fig. 1b. This unique loading geometry is clearly different from any indentation test in that the deformation force is applied in the transverse plane. Thus, the *ex vivo* loading geometry matches the *in vivo* set-up except that the boundary conditions are very different. Let us denote the original height of the sample as H_0 (the diameter of the cross-section), at measurement step i , the current height of the sample as H_i and the contact length between the sample and stamp (object plate) as L_i (Fig. 2). If we assume that the distance from the tissue center to the furthest contact point is still the original radius, i.e., $H_0/2$, then the contact length can be approximated as:

$$L_i \approx \sqrt{H_0^2 - H_i^2} \quad (1)$$

The contact area between the sample and pressing stamp, denoted by S_i , is therefore calculated as:

$$S_i = wL_i \approx w\sqrt{H_0^2 - H_i^2} \quad (2)$$

where w denotes the width of the stamp. As evidenced by finite element model (FEM) simulation results presented in the next section, this approximation is reasonable.

The stress σ is the ratio of the force to the contact area. The natural strain is calculated as accumulated fractional decrease in the sample height:

$$\varepsilon_i = \frac{H_{i-1} - H_i}{H_{i-1}} + \varepsilon_{i-1} \quad (3)$$

According to the Hooke's law, $\sigma = E\varepsilon$, where E is the Young's modulus of the sample. The device estimates instantaneous E as the local slope of the stress-strain curve using first-order forward numerical differentiation. To take into account nonlinear stress-strain relationships, we average the instantaneous Young's modulus within the measured strain range and call the resulting value the average Young's modulus.

Finite Element Simulations

The deformation behavior of a contacting cylinder compressed by two plates in the transverse plane is a classic elasticity problem discussed by Gladwell (1980). However, to the best of our knowledge, we are not aware of any analytical solution if the friction between the cylinder and plates has to be considered. Therefore, to evaluate our mechanical measurement system, we used a commercial finite element software package ANSYS (ANSYS corporation, Canonsburg, PA) to simulate a cylindrical sample under compression. The sample is modeled as elastic, linear, isotropic and quasi-incompressible. Most soft tissues are assumed to have a Poisson's ratio between $\nu=0.45$ and $\nu=0.49$ (Chen et al. 1996). Since thrombi may not be incompressible especially during the acute phase when serum or blood might be forced out of a thrombus during deformation, we modeled our tissue sample with a Poisson's ratio in a broader range of $0.40 < \nu < 0.49$.

For the rigid stamp and supporting plate, their Young's moduli were both set at $E=200$ GPa and the Poisson's ratio $\nu=0.27$ (typical values for iron and steel). The contact between the stamp (plate) and the sample was modeled using a "contact pair" (i.e., two surfaces that come into contact with each other by a load), where the stamp (plate) was defined as "target" and the sample was "contact" (ANSYS 7.1, 2004a). We assumed two extreme friction conditions between the sample and targets, i.e., friction coefficient = 0 and friction coefficient = ∞ (this is defined as rough friction contact) (ANSYS 7.1, 2004a). We fed an FEM simulation with a

known Young's modulus for the test sample and the analysis option was chosen as "large displacement static" model (ANSYS 7.1, 2004b). Known compression force was then exerted to the stamp in multiple incremental steps. At each step, the force induced tissue height change was recorded. The deformed axisymmetric element meshes from original height H_0 to height H_i are shown in Fig. 3. Finally, based on the force-displacement measurements, we applied the scheme introduced in the previous section to calculate the Young's modulus and evaluated it against the true value.

Animal Methods

Animal studies were performed using a rat model of stasis induced venous thrombosis (Wakefield et al. 1995). The protocol was approved by the University of Michigan Committee on the Use and Care of Animals and strictly complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 59 Sprague-Dawley rats were studied. On the first day of the experiment, all rat IVCs below the renal veins and their associated back branches were ligated. As a direct result of flow stasis within the venous circulation, thrombosis formation was initiated in the IVC. Rats were divided into two groups with 14 rats in the first group and remaining 45 in the second. The difference in numbers between the two groups was based on time restrictions which dictated a smaller number of animals in the first group where we both imaged thrombi as well as performed *ex vivo* mechanical measurements. Two rats from the first group were diagnosed with no thrombosis by ultrasound examination, and they were therefore exempted from the study. On day 3 following ligation, all 12 rats in the first group underwent ultrasound elasticity imaging experiments, at the end of which, four animals were euthanized for the mechanical measurements on IVC thrombi *ex vivo*. On days 6 and 10, ultrasound elasticity imaging was repeated on the remaining rats in group one, but four rats were sacrificed on each day for mechanical measurement *ex vivo*.

Unlike the first group, animals in the second group only participated in mechanical measurements. This group acted as a negative control where any influence of prior pushing on the thrombi during elasticity imaging could be assessed. These rats were measured in groups of 9 on days 3, 6, 10, 12 and 14. It turned out that 7 rats did not develop thrombosis in group two. The number of rats in the second group was based on a power analysis using data from the first group to detect a 10 KPa difference between day 3, day 6, and day 10 with $p < 0.05$ and 80% power. We could provide this statistical power given that we only performed mechanical measurements without imaging on these thrombi.

Comparisons between thrombi from days 3, 6, and 10 from the group 1 rats, whose thrombi were both imaged *in vivo* and mechanically measured *ex vivo*, and from the thrombi of corresponding age in group 2 rats, whose thrombi were only measured mechanically *ex vivo*, were made using a two-tailed t-test with unequal variances. Differences with $p < 0.05$ were considered significant.

To prepare mechanical measurement, animals were first euthanized in an operating room, and thrombi were removed from the dissected IVC. They were then placed in individual sample holders and transported immediately to the next room for mechanical measurement. To maintain the tissue moisture content, sample holders were immersed in a saline solution. Once the measurement is complete, thrombi were put back into the saline solution, and then magnified pictures were taken.

Both the *in vivo* and *ex vivo* Young's modulus estimates rely on cylindrical geometry. Obviously, these samples were from living tissue, and although they were generally cylindrical, there were often variations in radii across any given thrombus, particularly for the chronic thrombi (Fig. 6). In order to make our *ex vivo* mechanical measurements over portions of

thrombi with the most circular cross-section, locations for sampling were selected based on the consensus visual assessment of three independent observers.

Strain images

Overall, strain images were generated in the same way as described in the previous study (Xie et al. 2004). In brief, we used a Siemens Sonoline “Elegra” ultrasound scanner with a 12.0 MHz linear array transducer (VFX13-5) to obtain ultrasound data. Anesthetized rats were placed in a supine position with the transducer applied to the shaved abdominal surface. Steady, continuous tissue deformations were produced by manually translating the scanhead in one-dimension with a specially designed mechanical device. Six second static deformations in the transverse plane of the thrombus-containing IVC were generated. We typically acquired 174 frames of ultrasound in-phase and quadrature (IQ) data during the transducer motion. The IQ data was subsequently transferred to a PC for off-line processing.

In each data set, we employed a 2-D correlation-based phase-sensitive speckle tracking algorithm to estimate motion (Lubinski et al. 1999). First, with a kernel size approximately equal to one speckle spot (0.60 mm laterally by 0.17 mm axially), cross-correlation was calculated using the baseband complex signal. The cross-correlation function was then spatially lowpass filtered with a 1.20 mm (lateral) by 0.85 mm (axial) 2-D separable Hanning window and then fitted with a 2-D second-order polynomial function. Axial and lateral displacements were estimated at the correlation lag where the magnitude of the cross-correlation maximized. To further improve the accuracy of the axial displacements, the baseband correlation function was transformed into its analytic-signal-based counterpart by simply adding a linear phase term. The resulting correlation function’s phase zero-crossing position represented the axial displacement. In addition, the signal-to-noise ratio (SNR) was enhanced by accumulating incremental frame-to-frame displacements over a number of frames within the same deformation sequence. Finally, axial normal strains were calculated as the spatial derivative of the accumulated axial displacements with respect to the axial direction using centered numerical differentiation. Given that the deformation force varied across different days for different rats, we had to normalize the strain estimates. As described previously (Emelianov et al. 2002; Xie et al. 2004), this was accomplished by individually normalizing strain images by dividing by the magnitude of the average surface-applied strain for each deformation. The axial normal strain values in our experiment range between -1 and 0 with a negative sign representing compression, whereas the normalized strains themselves can take any non-positive number. Thrombi with normalized strains of less than -1 are softer than the surrounding tissue on average.

Young’s modulus reconstruction

In a well controlled animal study, strain alone is effective enough to age DVT with fairly high accuracy. However, normalization does not account for the effects of tissue geometry and non-uniform stress distribution. Further due to strain intensification at boundaries, elasticity images cannot be compared directly with *ex vivo* Young’s modulus measurements of thrombi. Hence, in order to make these comparisons, one needs to estimate the Young’s moduli of thrombi in rat IVCs.

Therefore, we developed a model-based Young’s modulus reconstruction algorithm for DVT. The thrombus-containing vessel was represented as a long elastic, incompressible cylinder with a circular cross-section (Aglyamov et al. 2004b). In cross-section, the modeled thrombus was composed of multiple concentric layers with a unique Young’s modulus assigned to each layer with elastic moduli being a function of radial position only. For this particular model, a theoretical solution of the strain distribution exists for a given deformation if prior knowledge of the Young’s modulus exists. By experimentally measuring the strain distribution using

speckle tracking during abdominal surface deformation and having the analytic solution to the forward problem, the inverse problem of Young's modulus reconstruction can be iteratively solved as an optimization problem.

Briefly, the method works by performing successive approximations of the radially symmetric Young's distribution in a thrombus, calculating the relative strains one would get given that distribution, and then calculating the difference between the theoretical strain distribution and the experimentally acquired strain distribution. The local relative differences represent an error measure that can be minimized by altering the relative Young's moduli in the rings and the numbers of the rings. This process is repeated until the error is minimized, which here is taken as the relative difference change between two successive iterations being less than 2%. The Young's modulus distribution minimizing the error between experimentally measured and analytically predicted strains over a region of interest is accepted as the final solution. The reliability and efficiency of our reconstruction algorithm has been evaluated using numerical simulation and experiments with tissue-mimic phantoms. The algorithm was described in detail in previous publications (Aglyamov et al. 2004a; Aglyamov et al. 2004b).

Without knowledge of the deformation force or stress, thrombus Young's modulus is reconstructed with respect to that of the venous wall using our model. The Young's modulus of the vessel wall is defined as unity. So in order to compare them with direct mechanical measurements, we scale the reconstruction results by a constant factor so that both *in vivo* and *ex vivo* have the same absolute modulus on day 3, which is time of our first *ex vivo* measurements.

RESULTS

Validation of MicroElastometer by FEM simulation

For a cylindrical sample with original diameter 2.4 mm, we input $E=5.0$ KPa and $\nu=0.49$ to the FEM forward problem simulation. Based on the force-displacement measurement, we use the proposed approximation method to calculate the Young's modulus. The estimated value is $E=5.90$ KPa averaged in the natural strain range $[0, 50\%]$ for the case of frictionless sliding contact. In Fig. 4, the contact length between the target and the stamp is calculated during simulated deformation and compared against the one obtained from the approximation in eqn. (1). There is underestimation and overestimation in the contact length by eqn.(1) within the strain range. The maximum relative error between the true contact length and the approximation is constrained to 25%, nevertheless, the relative error between the true Young's modulus and the estimated value is about 18%. With the friction coefficient fixed at value 0, we varied the Poisson's ratio ν from 0.40 to 0.49, the resulting relative error between the true Young's modulus and the estimates range from -1% to 18%. We also investigated how the friction coefficient affects Young's modulus estimation. At $\nu=0.49$, the estimated Young's modulus is 5.90 KPa and 6.40 KPa for the case of frictionless surface contact and rough surface contact respectively. As ν goes down to 0.40, the Young's modulus is 4.95 KPa and 5.03 KPa for the two surface contact cases, indicating the friction effect is almost negligible. FEM simulation results verified that our proposed scheme provides a reasonable approximation to the true Young's modulus. Considering the combined effects of the Poisson's ratio and the extreme friction condition, the maximum relative error is overestimation by about 28%.

In Fig. 5, a simulated force-strain curve is compared to a measured curve for a calibration rubber phantom with a known Young's modulus $E=86$ KPa, Poisson's ratio $\nu=0.49$ and original diameter $D=2.8$ mm. The nonlinearity between the force and strain is mainly contributed by the geometric properties of the linear test sample. Clearly, the simulation agrees closely with the measurement, which further verifies the device itself.

Animal Experiments

Fig. 6 presents pictures of thrombi on days 3, 6, 10 and 12 respectively. In addition to size reduction, inhomogeneity in thrombus appearance is clearly observed as thrombi progress from acute to chronic stages. This is due to the replacement of blood cells, platelets and inflammatory cells in acute thrombi with collagen and fibrin in chronic thrombi, which is believed to be the main mechanism of thrombus hardening (Browse et al. 1999).

For rat #12 in the first group, B-scan images along with their corresponding normalized strain maps over a region of interest (ROI) are shown in Fig. 7 for three scanning days. The physical dimensions of the ROI are 4.5 mm laterally by 4.5 mm axially. It centers at the thrombus and encompasses the entire IVC, part of the adjacent aorta (anatomic left of the IVC, which is on the right side in the transverse image) and the immediate surrounding tissue. In the area close to the aorta, the speckle pattern decorrelates very rapidly due to the aorta motion, resulting in poor displacement estimates and strain estimates. The normalized strain images are displayed over a dynamic range from -1.9 to -0.4 , in which black represents relatively soft tissue and white represents relatively hard tissue. The minus sign represents compression in the axial direction. The consistent decrease in normalized strain magnitude is obviously observed, indicating thrombus hardening follows age.

Figure 8 compares measured stress-strain curves for a typical 3d old thrombus and a 10d old thrombus. Clearly, the 10d old thrombus is not only much harder than the 3d old, but it also exhibits significantly more elastic nonlinearity. To assure consistent contact between the pressing stamp and the thrombus samples at the onset of deformations, measurement points were considered invalid if their associated force is less than 10% of the maximal pressing force. Those points with the natural strain greater than 50% were also discarded from Young's modulus estimation due to high deformability of the sample. The estimated average Young's moduli are summarized in Table 1 for two groups. None of the differences between the two groups of rats at days 3, 6, and 10 were significant ($p > 0.05$). This consistency between two groups suggests that *in vivo* compression applied during ultrasound elasticity imaging imposes no remarkable change to the elastic property of thrombi, at least from day 3 and later. It follows that the hardness change monitored by the imaging experiment reflects the true thrombus hardening process.

In Fig. 9, relative Young's modulus reconstructed from four animals is plotted as function of thrombus age. Clearly, the Young's modulus increases with thrombus aging, which is consistent with decreasing strains in elasticity imaging. Then, as mentioned above, we used the *in vivo* reconstructions for comparison with the *ex vivo* measurements of Young's modulus. For the rat model, transition from acute to chronic status is believed to occur around day 6–7, and day 10 corresponds to well into the chronic phase. This is suggested by the evidence that the predominant leukocytes of inflammatory reaction in rat IVC walls with surgically induced thrombi converts from acute neutrophils to more chronic monocytes/macrophages in about 6 days (Wakefield et al 1995). As illustrated by Fig. 9, reconstruction agrees well with the mechanical measurement at early days. The major discrepancy between the two methods occurs as thrombi develop into the chronic stage.

DISCUSSION AND CONCLUSIONS

The major concern of DVT diagnosis is to identify acute thrombi so that they can be treated with heparin to prevent PE promptly. Good agreement between the mechanical measurement and reconstruction up to the chronic stage certainly assures the reliability of elasticity imaging to age acute DVT.

As for the discrepancy existing during the chronic stage, one main explanation might be the perfect cylinder model assumed by both reconstruction and mechanical measurement. This assumption is less valid as thrombi become non-occlusive and chronic, as indicated by magnified photographs of thrombi. Variations from the model will invalidate the theoretical solution in reconstruction and contact area calculation in mechanical measurement, potentially leading to incorrect Young's modulus estimates. This explains why mechanical measurements have the highest variation on day 14 in Fig. 9. The effect of boundary conditions may also contribute to the discrepancy. Whereas thrombi are measured laterally unconstrained by the mechanical device, ambiguity in separating surrounding tissue and vessel walls in reconstruction will influence the elastic contrast between the thrombus and the wall. Another possible reason to explain the discrepancy is the strain range. Using a mechanical device on *ex vivo* thrombi, it is easy to control the deformation range. However in our elasticity imaging experiment, comparison is based on the same 15%–20% average surface applied strain (the relative position change of the spine to the abdominal surface) on top of some unknown preload. At this level, the internal thrombus strain is different at different ages. As shown in Fig. 8, Young's modulus has a nonlinear relationship with the strain, so the value varies depending on the strain level. Finally, the ultrasound Young's modulus reconstruction is still preliminary given a relatively small number of rats involved. It is likely that part of the discrepancy can be resolved with more test samples. It should be said, however, that these discrepancies between the model and thrombi are occurring when thrombi are clearly chronic. Estimates of age are much less critical during this period, since chronic DVT have a very low propensity to embolize. The vital determination is to call acute DVTs acute so that they can be appropriately treated. For these acute thrombi, the concordance between model and experiment is very high.

In summary, we developed a mechanical measurement system to characterize the elastic property of *ex vivo* thrombi. By FEM simulation, we validated the device as well as the scheme proposed to estimate the Young's modulus for cylindrical test samples. Based on a well established rat-model of DVT, *ex vivo* mechanical measurements were carried out for two groups of animals. Prior to mechanical measurement, thrombi developed in group one were monitored successively by ultrasound elasticity imaging. No elasticity imaging experiment was performed on group two. Our results show the consistency between the Young's moduli measured from two groups suggesting that deforming thrombi *in vivo* does not significantly change their elastic moduli, at least from day 3 and later. Direct mechanical measurements were further compared to the relative reconstruction results. This comparison can be viewed as a calibration between elasticity measurements for aging DVT and the true Young's moduli of thrombi. As shown in Fig. 9, the good agreement of the means for day 6 thrombi and the overlap of the standard deviations for day 10 thrombi conservatively suggest a level of agreement between strain measurements and true Young's modulus estimates that adds more support to the ability of ultrasound elasticity imaging to age DVT.

Acknowledgements

This study was supported by NIH grant HL-68658. We thank Siemens, Inc. for equipment and technical support. We also thank Marisa Linn and Angela Hawley from the Vascular Surgery Laboratory, University of Michigan for their assistance with animal care.

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Fig. 1. Photos of the mechanical measurement device-MicroElastometer: (a) full view, (b) illustration of the loading geometry of a cylindrical phantom.

Fig. 2. Schematic illustration of how the contact area is approximated during sample deformation. Sample on the left is in its original shape with height H_0 , and sample on the right is under deformation with height H_i and contact length L_i .

Fig. 3.
The finite element meshes of a test sample under deformation when the tissue height changes from original H_0 to H_i (ANSYS simulation).

Fig. 4. Simulated contact length during deformation is compared with the approximated value based on Eq. (1). The dashed line is the response if the approximation perfectly agrees with the simulation.

Fig. 5. Measured force-strain curve is compared to the simulated curve for a cylindrical phantom (diameter 2.8 mm) with a known Young's modulus $E=86$ KPa.

Fig. 6. Magnified pictures of thrombi at different ages: (a) 3-day, (b) 6-day, (c) 10-day and (d) 12-day.

Fig. 7. Thrombus of rat #12 in the first group over three scanning days. Top row: B-Scan images (dynamic range 50 dB) over a $4.5 \text{ mm} \times 4.5 \text{ mm}$ ROI; bottom row: normalized strain images (on a scale -1.9 to -0.4). The clot boundary is approximately outlined by a dotted-lined ellipse. Thrombus hardening correlates with the gradual color change from black to white within the thrombus in the strain images.

Fig. 8.
Measured stress-strain curves obtained from a 3-day old thrombus and a 10-day old thrombus.

Fig. 9. Young's modulus increases as thrombus ages. The *ex vivo* direct mechanical measurements are compared to the results obtained from *in vivo* Young's modulus reconstruction. The error bar represents $\pm$ standard deviation.

Table 1

The mean and standard deviation of the measured average Young's modulus (KPa) for thrombi at different ages. The differences between the two groups at days 3, 6, and 10 are not significant.

	Day 3	Day 6	Day 10	Day 12	Day 14
Group I	8.53 ± 2.66	17.35 ± 4.62	34.73 ± 8.26		
Group II	9.46 ± 0.87	17.40 ± 5.77	38.41 ± 9.17	70.42 ± 16.21	82.83 ± 37.78