Biaxial Tensile Properties of Thoracic Aortic Aneurysm Tissues*

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Abstract
Mechanical properties of human aortic aneurysm tissues were measured with a biaxial tensile tester. Fifteen-mm-square specimens were obtained from thoracic aortic aneurysms of various origins and from undilated aortas adjacent to the aneurysms during aneurysmectomy, and were stored frozen until the measurement. Each specimen was stretched biaxially in physiological saline at room temperature at the rate of ~0.2 mm/sec. Although the ordered displacement was set equal for both directions, real strain applied to the specimens was not equibiaxial. The stress-strain curves under equibiaxial stretch were obtained by fitting measured curves with a strain energy function considering material anisotropy. Effects of freezing and ambient temperature on the mechanical properties were evaluated with porcine thoracic aortas. The mechanical properties of the frozen-stored specimens at 23°C were almost similar to those of the fresh specimens at 37°C. Elastic modulus at zero load averaged for both directions $H_m = (H_x + H_y)/2$ was higher ($P < 0.01$) in the aneurysm tissues (1450 ± 250 kPa, mean ± SEM, $n = 26$) than in the undilated tissues (650 ± 140 kPa, $n = 10$). Anisotropy index $K = |H_x - H_y|/H_m$ was not significantly different between the aneurysm (20 ± 3%) and the undilated tissues (12 ± 3%) for all specimens. For the specimens whose elastic modulus $H_m$ was smaller than 1 MPa, however, the index $K$ was significantly higher ($P < 0.05$) in the aneurysm specimens (23.1 ± 5.3%, $n = 14$) than the undilated tissues (9.5 ± 2.5%, $n = 8$). These results indicate aneurysm tissues are not only stiffer but also more anisotropic than the nonaneurysmal tissues.

Key words: True Aneurysm, Dissecting Aneurysm, Annulo Aortic Ectasia, Post-Stenotic Dilatation, Marfan's Syndrome, Mechanical Properties, Tensile Test, Anisotropy, Elastic Modulus

1. Introduction
Rupture of thoracic aortic aneurysm is fatal in many cases. Its mortality rate has been reported to be more than 90% (1). While aortic graft replacement surgery is recommended when the aortic diameter exceeds 5–6 cm, some of the aneurysms rupture at smaller diameters, and others do not rupture at larger diameters. Accurate prediction of the rupture and careful planning of the treatments play a key role in reducing the risk of patients as well as medical cost. Thus, several groups have started finite element analyses of the
aneurysms aiming at the prediction of the aneurysm rupture (2–6). Although precise knowledge on the mechanical properties of the aneurysm tissues is very important for such investigations, most of the studies have used mechanical properties obtained in the uniaxial tensile test (7–10).

Generally speaking, aneurysms were spherical in shape. This indicates that aneurysm tissues are stretched almost equibiaxially due to blood pressure in the in vivo state. There is no guarantee that results obtained from a uniaxial test can be applied to the biaxial state. In this study, we have developed a 2D tensile tester and obtained the mechanical properties of aneurysm tissues of various origins under a biaxial stretch condition.

2. Materials and Methods

2.1 Specimen

Specimens were obtained from thoracic aortic aneurysms during aortic replacement surgery at Tohoku University Hospital, as approved by the Tohoku University Hospital Human Studies Committee according to World Medical Association Declaration of Helsinki. Patients gave informed consent prior to surgery. The total of 26 specimens were obtained from 16 patients with various diseases (Table 1). Ten nonaneurysmal specimens were also obtained from 5 undilated aortas adjacent to the aneurysms as control. Aneurysm specimens were obtained at around maximal diameter position of each aneurysm. The specimens were rectangular with edges aligned to circumferential and longitudinal directions. They were immersed in the physiological saline and stored frozen at –20°C until measurement.

Table 1  Summary of specimens and their mechanical properties. (mean±SEM)

<table>
<thead>
<tr>
<th>Origin</th>
<th>n/N As/Ar/Ds</th>
<th>$\lambda_x^e$</th>
<th>$\lambda_y^e$</th>
<th>$\sigma_x^e$ (kPa)</th>
<th>$\sigma_y^e$ (kPa)</th>
<th>$\sigma_{in vivo}$ (kPa)</th>
<th>$H_{mi}^{eqvi}$ (kPa)</th>
<th>K (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>10/5</td>
<td>1.11±0.03</td>
<td>1.08±0.03</td>
<td>185±47</td>
<td>190±51</td>
<td>50±9</td>
<td>652±140</td>
<td>12.4±3.1</td>
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<tr>
<td>TA</td>
<td>13/9</td>
<td>1.09±0.02</td>
<td>1.08±0.02</td>
<td>308±98</td>
<td>266±72</td>
<td>93±12</td>
<td>1617±391*</td>
<td>22.1±5.6</td>
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<td>DA</td>
<td>6/3</td>
<td>1.09±0.03</td>
<td>1.05±0.02</td>
<td>317±95</td>
<td>279±86</td>
<td>55±11</td>
<td>1383±584</td>
<td>19.3±5.9</td>
</tr>
<tr>
<td>AAE</td>
<td>4/2</td>
<td>1.17±0.02</td>
<td>1.12±0.01</td>
<td>615±87</td>
<td>496±65</td>
<td>104±28</td>
<td>1686±581</td>
<td>11.8±3.4</td>
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<tr>
<td>PSD</td>
<td>2/1</td>
<td>1.28±0.03</td>
<td>1.22±0.02</td>
<td>568±9</td>
<td>530±38</td>
<td>75±15</td>
<td>450±22</td>
<td>19.8±17.6</td>
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<tr>
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<td>1/1</td>
<td>1.21</td>
<td>1.16</td>
<td>709</td>
<td>534</td>
<td>94</td>
<td>709</td>
<td>26.6</td>
</tr>
</tbody>
</table>

*P<0.05 vs NM. NA, nonaneurysmal; TA, true aneurysm; DA, dissecting aneurysm (whole wall adjacent to the dissecting site); AAE, annulo aortic ectasia; PSD, post-stenotic dilatation; MS, Marfan’s syndrome. n/N, number of specimens/patients; As/Ar/Ds, number of specimens obtained from ascending aorta/aortic arch/descending thoracic aorta; $\lambda_x^e$, stretch ratio at the end of the test in direction n; $\sigma_x^e$, stress at the end of the test in direction n; $\sigma_{in vivo}$, in vivo stress estimated from the law of Laplace; $H_{mi}^{eqvi}$, initial elastic modulus averaged for directions x and y; K, anisotropy index. See sections 2.4–6 for details of parameters on the mechanical properties.

2.2 Biaxial Tensile Tester

Biaxial tensile testing was performed with a biaxial tensile tester developed in our laboratory (Fig.1(a)). A specimen is connected to two sets of arms and stretched in the two orthogonal directions simultaneously. Each set of the arms is mounted on a linear guide with an oppositely threaded screw driven by a stepping motor. Each set of arms has a laboratory-made cantilever-type load cell at each tip to measure tensile load applied to the specimen (Fig.1(b)). The load cell was fabricated by applying two strain gages on each side of a stainless steel plate of 30x15x0.5 mm. The resolution of the cantilever was 10 mN. Specimen deformation was measured with a video dimension analyzer (Percept scope, Hamamatsu Photonics, Japan) on the images taken with an overhead CCD video camera. Data measurement and control of the tester were done with LabVIEW ver.5.0.
The spatial resolution of the dimension measurement was smaller than 15 µm in the vertical direction of the screen.

Fig. 1  Schematic representation of the 2 dimensional tensile test system (a) and the details of specimen chucking (b).  VDA, video dimension analyzer.

2.3 Measurement

Specimens were thawed and a rectangular piece with 15 mm square was cut out from each specimen with edges aligned to circumferential and longitudinal directions. When the directions along which the specimens were taken were known, the circumferential and axial directions were taken as x and y directions, respectively. The directions were taken arbitrarily in other cases, i.e., x direction was taken as circumferential or axial direction. Specimen thickness $h_0$ was measured with a dial gauge with the resolution of 10 µm. A 3-mm thick aluminum plate of 6 x 7 mm was attached to the probe tip to prevent indentation of the tip into the specimens. The thickness was measured at three different positions and averaged. A 5 mm square bench mark was then drawn in the middle of the specimen with black shoe polish and the actual distance between its opposite sides in x and y directions, $l_{x0}$ and $l_{y0}$, respectively, were measured with a caliper. Each edge of the specimen was stuck with four or five small hooks at regular intervals on the line 1.5 mm from the rim, and the hooks were connected to each carriage of the tester with a silk thread (2-0) continuously like a trampoline (Fig.1(b)). We used different number of hooks in x and y directions due to technical difficulties. This may cause different mechanical conditions between directions. Possible effect of this difference was compensated by considering a stress correction factor when calculating stress in x and y directions (See equation (2)). The distance between the opposite hooks in x and y directions, $L_{x0}$ and $L_{y0}$, respectively, were measured similarly with the caliper. The specimen was then stretched biaxially in a physiological saline at room temperature by widening the distance between the opposite arms at the rate of 0.2 mm/s, which corresponds to the strain rate of 1.7%/s, while measuring the distance between the opposite sides of the bench mark in x and y directions, $l_x$ and $l_y$, respectively, and the tensile load in x and y directions, $F_x$ and $F_y$, respectively. The tensile test was started at the point where the specimen had 0.1 to 0.2N tension in each direction. All of the specimens were stretched without preconditioning because some of them could be very fragile. The test was terminated when the specimen began tearing at the hooks.

To compare the mechanical properties obtained in the biaxial test with those in the conventional tests, uniaxial properties were measured for four specimens before the biaxial test. After the specimens were set to the tester, they were stretched in the x direction at the
rate of 0.2 mm/s, while the threads in the \( y \) direction were relaxed fully throughout the test. Then the mechanical properties in the \( y \) direction were measured similarly. To prevent plastic deformation of the specimens during uniaxial test, much attention was paid not to overload specimens.

### 2.4 Calculation of stress and strain

Green strain in the \( x \) and \( y \) directions (\( E_x, E_y \)) and Cauchy stress in these directions (\( \sigma_x, \sigma_y \)) were calculated from the load applied to the specimen (\( F_x, F_y \)), distance between the benchmarks (\( l_x, l_y \)) at that point, and the thickness \( h_0 \) in the no load state assuming the incompressibility of the specimen. Green strains were obtained as

\[
E_x = (\lambda_x^e - 1)/2 = (l_x/l_{x,0})^{1/2} - 1/2, \quad E_y = (\lambda_y^e - 1)/2 = (l_y/l_{y,0})^{1/2} - 1/2
\]

where \( \lambda_n \) is stretch ratio in the \( n \) direction. Cauchy stresses were calculated as

\[
\sigma_x = k \frac{F_x}{h_0 l_{x,0}} \lambda_x^e, \quad \sigma_y = k \frac{F_y}{h_0 l_{y,0}} \lambda_y^e,
\]

where \( k \) is a stress correction factor. In a pilot study with a finite element analysis, we found that the stress calculated assuming that the tensile load applied uniformly to the line on which the hooks were attached to the specimen (\( k=1 \) in Eq.2) was about 10% larger than the mean stress in the area of the square benchmark. The factor was for such correction and was 0.90 for the 5-hook side and 0.89 for the 4-hook side.

### 2.5 Estimation of stress-strain curves in the equibiaxial strain state

Although the displacement rate of the arms was set equal for both directions, strain applied to the specimen was not equal, because of the elongation of the thread, the deflection of the cantilever, the distance between the opposite hooks, etc are different between the two directions. To obtain the stress-strain curves in the equibiaxial strain state, we fitted a strain energy density function to the raw data and then obtained the stress-strain curves under biaxial stretch theoretically.

As the strain energy density function, we used Fung’s exponential type two-dimensional strain energy function considering material anisotropy\(^{(11)}\):

\[
W = \frac{C}{2} \exp(a E_x^2 + b E_y^2 + 2c E_x E_y)
\]

where \( E_x \) and \( E_y \) are the Green strains in \( x \) and \( y \) directions, respectively, and \( C, a, b, \) and \( c \) are the material constants obtained for each specimen. Stress-strain relationships were derived as:

\[
\sigma_x' = \lambda_x^e \frac{\partial W}{\partial \lambda_x} = (2E_x + 1)(a E_x + c E_y)C \exp(a E_x^2 + b E_y^2 + 2c E_x E_y)
\]

\[
\sigma_y' = \lambda_y^e \frac{\partial W}{\partial \lambda_y} = (2E_y + 1)(b E_y + c E_x)C \exp(a E_x^2 + b E_y^2 + 2c E_x E_y)
\]

The material constants were determined to minimize the error between the curve and the raw data:

\[
err = \sum |\sigma_x' - \sigma_x'| + \sum |\sigma_y' - \sigma_y'| \rightarrow \text{min.}
\]

Stress-strain relationships in the equibiaxial strain state (\( E_x = E_y = E \)) are obtained as:

\[
\sigma_x'^{\text{eq}} = (2E + 1)E(a + c)C \exp[(a + b + 2c)E^2]
\]

\[
\sigma_y'^{\text{eq}} = (2E + 1)E(b + c)C \exp[(a + b + 2c)E^2]
\]
where the superscript equi denotes equibiaxial condition. With regard to the accuracy of such estimation, we performed a pilot study, in which we stretched normal porcine thoracic aortas at various combinations of strain rates and found that if the difference in the strain between the two axes was smaller than 50% then the estimations did not differ statistically from the data obtained under equibiaxial condition.

2.6 Mechanical parameters used for the analysis

Elasticity of aneurysmal wall was evaluated with an initial elastic modulus, i.e., elastic modulus at zero load, in x and y directions, \( H_{\text{xequ}} \) and \( H_{\text{yequ}} \), respectively, under equibiaxial stretch. They are expressed with the material constants as follows:

\[
H_{\text{xequ}} = \frac{d\sigma_{\text{xequ}}}{dE} \bigg|_{E=0} = C(a + c), \quad H_{\text{yequ}} = \frac{d\sigma_{\text{yequ}}}{dE} \bigg|_{E=0} = C(b + c). \tag{7}
\]

Their mean value \( H_{\text{mequ}} \) was used as the index of specimen elasticity:

\[
H_{\text{mequ}} = \left( H_{\text{xequ}} + H_{\text{yequ}} \right)^{\frac{1}{2}} = C(a + b + 2c)/2. \tag{8}
\]

Wall anisotropy was evaluated with normalized difference in the elastic modulus between the two directions \( K \):

\[
K(\%) = \frac{\left| H_{\text{xequ}} - H_{\text{yequ}} \right|}{\left( H_{\text{xequ}} + H_{\text{yequ}} \right)^{\frac{1}{2}}} \times 100 = \frac{2|a - b|}{(a + b + 2c)}. \tag{9}
\]

2.7 Statistical analysis

Data are expressed as mean±SEM. Differences were analyzed by the Student’s t-test and were considered significant when \( P < 0.05 \).

3. Results

3.1 Specimens

Most of the aneurysm specimens were affected with atherosclerosis (Fig.2). Their luminal surface was not smooth (a). Fragmentation and disappearance of elastic lamella (#31, 38, 46) and accumulation of collagen (#31, 38, 53, 46) were prominent (b).

The type of disease that caused the aneurysm and the number of specimens used in the present study are summarized in Table 1. Their stretch ratios, \( \lambda_{\text{xe}} \) and \( \lambda_{\text{ye}} \), and stresses, \( \sigma_{\text{xe}} \) and \( \sigma_{\text{ye}} \) at the end of the test along with their mechanical properties are also shown along with in vivo stress \( \sigma_{\text{in vivo}} \) estimated from mean blood pressure, aneurysm diameter, and thickness of aneurysmal wall by applying the law of Laplace for a sphere. All the specimens tore at the hooks and \( \lambda_{\text{xe}} \) and \( \lambda_{\text{ye}} \) were relatively low compared to physiological strain of the healthy arteries. However, \( \sigma_{\text{xe}} \) and \( \sigma_{\text{ye}} \) were much higher than \( \sigma_{\text{in vivo}} \). Because the stretch ratio was lower than 1.05 in either direction in 15 out of 36 specimens, we evaluated the elastic properties of the aneurysmal wall with the initial elastic moduli. The initial elastic modulus was significantly higher in the true aneurysm than in the nonaneurysmal tissue. With regard to the anisotropy index, there was no significant difference among the origins of the specimens. The number of the specimens whose in situ direction was known clearly was seven for nonaneurysmal, eight for true aneurysm, two for dissecting aneurysm, and one for Marfan’s syndrome specimens. Although 12 out of 18 specimens were stiffer in the circumferential direction, the difference between the circumferential and axial directions was not significant. With regard to the nonaneurysmal specimens, 6 out of 7 specimens were stiffer in the circumferential direction. However, the difference between the directions was not significant either at the \( P \) value of 0.05. Nonaneurysmal, true and dissecting aneurysm
specimens were obtained from multiple locations. No significant correlation was observed between the mechanical parameters and the locations.

The dimensions and material constants of representative specimens of each origin are shown along with age, sex, and blood pressure of their hosts (Table 2). Each of the material constants had wide variation among specimens having similar mechanical properties, because similar stress-strain relationship could be fitted with different combinations of material constants. Thus, material constants averaged for each group do not well represent that group. Taking this into account, we showed the data of a representative specimen of each origin whose $H_{mi}$ and $K$ are close to the average of each origin.

![Specimens after stretch](image1)

(a) Specimens after stretch

![Histological sections](image2)

(b) Histological sections

Fig. 2 Examples of specimens after stretch (a) and histological sections stained with Elastica-Masson (b). Black squares in (a) are the benchmarks on the luminal surface. Luminal side faces downward in (b). See legend to Table 2 for abbreviations.

Table 2 Dimensions and material constants of a representative specimen of each origin along with age, sex, and blood pressure of their hosts.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Position</th>
<th>Age</th>
<th>Sex</th>
<th>mBP</th>
<th>$D$</th>
<th>$h_0$</th>
<th>$C$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$err$</th>
<th>$H_{mi}^{eq}$</th>
<th>$K$</th>
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<tbody>
<tr>
<td>NA</td>
<td>Ar</td>
<td>74</td>
<td>m</td>
<td>83</td>
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<td>2.1</td>
<td>5.8</td>
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<td>11.9</td>
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<td>709</td>
<td>26.6</td>
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</table>

NA, nonaneurysmal; TA, true aneurysm; DA, dissecting aneurysm; AAE, annulo aortic ectasia; PSD, post-stenotic dilatation; MS, Marfan's syndrome; Ar, aortic arch; As, ascending aorta; Ds, descending thoracic aorta; mBP, mean blood pressure; $D$, aneurysmal diameter; $h_0$, wall thickness; $C$, $a$, $b$, and $c$, elastic moduli in Fung's strain energy density function; $err$, fitting error per data point; $H_{mi}^{eq}$, initial elastic modulus averaged for directions $x$ and $y$; $K$, anisotropy index. See text for details.
3.2 Significance of the fitting

Stress-strain curves for the equibiaxial strain state could be very different from those obtained in the experiment (Fig.3). The raw stress-strain curves look very anisotropic in one case (Fig.3(a) upper). However, this happens because the stretch ratio at the same time point is different between the axes. If the curves are converted to these under the equibiaxial strain state (Fig.3(a) lower), it turns out that this specimen is not anisotropic. Another specimen looks isotropic in the raw curves (Fig.3(b) upper); it is somewhat anisotropic in the equibiaxial strain state (Fig.3(b) lower). Thus, it is very important to convert the curves for the equibiaxial condition.

![Fig. 3](image_url)

(a) #51-1 (50y, male, post-stenotic dilatation)  (b) #38-1 (74y, male, true aneurysm)

3.3 Comparison with uniaxial stretch

The mechanical properties of a specimen obtained from the present biaxial test and those from the conventional uniaxial test were almost similar to each other (Fig.4). The initial elastic modulus $H_i^0$ was 260 kPa for the uniaxial test and 353 kPa for the biaxial test. If the specimen is isotropic and homogeneous, the elastic modulus obtained in the biaxial test is twice the modulus obtained in the uniaxial test. However, the elastic moduli obtained in the two tests were almost similar to each other. This was also the case for all four specimens tested for the difference between the uniaxial and biaxial tests (Table 3). This indicates that biaxial tensile properties cannot be estimated from the uniaxial test.

Uniaxial stretch was performed before the biaxial test. In the uniaxial test, specimens were stretched in the $x$ direction first and then in $y$ direction. Although attention has been paid not to overload specimens during the uniaxial test, the maximum stress applied to the specimen was ~70% of stress at failure in the worst case ($y$ direction in Fig. 4). Considering we did not precondition the specimens, a precedent test may affect the results in the following test. However, no systematic tendency was observed in the measured parameters (Table 3), and these biaxial data were not outliers in terms of initial elastic modulus vs anisotropy index relationship (Fig.5). Thus, we believe that the effects of precedent tests were minor.
Fig. 4  Comparison of stress-stretch ratio curves obtained in the biaxial and uniaxial tensile test.

Table 3  Comparison of the initial elastic moduli (kPa) obtained from the present biaxial and the conventional uniaxial tests.

<table>
<thead>
<tr>
<th>Specimen #</th>
<th>Origin</th>
<th>Biaxial test</th>
<th>Uniaxial test</th>
</tr>
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<tbody>
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<td></td>
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<td>$H_{y_1}^{eq}$</td>
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<tr>
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<td>810</td>
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</table>

NA, nonaneurysmal; TA, true aneurysm, DA, dissecting aneurysm. $H_{x_1}^{eq}$ and $H_{y_1}^{eq}$, initial elastic modulus in direction $x$ and $y$, respectively; $H_{x_3}^{eq}$, initial elastic modulus averaged for directions $x$ and $y$.

3.4 Elastic properties under biaxial stretch

The initial elastic modulus was significantly ($P < 0.01$) higher in the aneurysm tissues ($1450 \pm 250$ kPa, mean $\pm$ SEM, $n = 26$) than in the nonaneurysmal tissues ($650 \pm 140$ kPa, $n = 10$). Anisotropy index was also higher in the aneurysm tissues ($19.9 \pm 3.1\%$) than in their counterparts ($12.4 \pm 3.1\%$), but the difference was not significant at the $P$ value of 0.05.

The relation between the anisotropy index and the initial elastic modulus was not clear on first glance (Fig.5). There was no significant correlation between the two parameters for all specimens and for specimens from any origin. However, data points of the nonaneurysmal specimens tend to locate closer to the origin: seven out of 10 nonaneurysmal specimens were within the range of $H_{x_3}^{eq} < 1000$ kPa and $K < 20\%$, while the aneurysm tissues in this range was less than 30% (7 out of 26 specimens). The anisotropy index $K$ of the aneurysm specimens whose elastic modulus $H_{x_3}^{eq}$ was smaller than 1000 kPa was $23.1 \pm 5.3\%$ ($n = 14$) and significantly larger than that of the nonaneurysmal tissues ($9.5 \pm 2.5\%$, $n = 8$, $P < 0.05$). Similarly, the elastic modulus $H_{x_3}^{eq}$ of the aneurysm specimens whose anisotropy index $K$ was smaller than 20% was $1446 \pm 317$ kPa ($n = 15$) and significantly larger than that of the nonaneurysmal tissues ($608 \pm 141$ kPa, $n = 8$, $P < 0.05$). These results indicate that aneurysm tissues may become stiffer or more anisotropic than the nonaneurysmal tissues.
4. Discussion

Mechanical properties of aortic aneurysmal walls have been evaluated in many studies both in vivo and in vitro. Most of the studies covered abdominal aortic aneurysm and pointed out that aneurysm walls are stiffer than their normal counterparts. In most of the in vivo studies, changes in the dimensions of the abdominal aneurysm were measured during a cardiac cycle with various ultrasound techniques to obtain its stiffness in the physiological state\(^{(12,13)}\). Neither stress-strain relationship nor anisotropy can be obtained in in vivo studies due to technical difficulties. Stress-strain relationships were measured in in vitro studies, but most of them used a uniaxial test\(^{(7–10)}\). Aneurysm tissues are, however, stretched biaxially in vivo, and in order to obtain detailed stress-strain relationship, their mechanical properties should be obtained by a biaxial test. Okamoto et al.\(^{(14)}\) measured the mechanical properties of dilated human ascending aorta under equibiaxial stretch. They obtained mechanical properties of ascending aortas of patients with various ages undergoing aortic replacement surgery and found that the stiffness increased with age. They also found that the degree of anisotropy decreased with the unloaded wall thickness, but had no significant correlation with age. Vande Geest et al. obtained similar results for abdominal aorta\(^{(15)}\). These data were obtained from specimens with few or no atherosclerotic lesions, and do not represent the mechanical properties of typical aneurysm specimens. On the contrary, we measured the biaxial tensile properties of aneurysm tissues caused by various diseases, and found that aneurysm tissues were stiffer or more anisotropic than nonaneurysmal tissues in terms of their initial elastic modulus (Fig.5). Many of the aneurysm specimens had rough luminal surface, and fragmentation and disappearance of elastic lamella and accumulation of collagen were prominent (Fig.2).

The change in the elastic modulus and the degree of the anisotropy in the aneurysm specimens might also depend on the type of the disease. As shown in Table 1, true aneurysm specimens were significantly stiffer than the nonaneurysmal ones. The specimens obtained from dissecting aneurysm and annulo aortic ectasia also tended to be stiffer. The differences were, however, insignificant, possibly due to the small number of the specimens. On the contrary, specimens obtained from post-stenotic dilatation and Marfan’s syndrome were not stiffer than the nonaneurysmal tissues at all. With regard to the anisotropy index, there was no significant difference among the origins, although it
tended to be higher in the aneurysm specimens than in the nonaneurysmal ones. However, this is not the case for the specimens with annulo aortic ectasia. We need to accumulate the elastic properties of aneurysm tissues of various origins.

Are aneurysm tissues really more anisotropic than the normal aortic tissues? It is hard to answer this question at this moment, because the results have been inconclusive. Raghavan et al. (8) did not find anisotropy in the abdominal aortic aneurysm tissues while Thubrikar et al. (9) did. With regard to thoracic aortic aneurysm tissues, Okamoto et al. (14) and Vorp et al. (10) did not find anisotropy while we did in the present study. There seems to be no systematic difference between the thoracic and abdominal aneurysms, although there are some differences between the thoracic and abdominal aortas, e.g., higher incidence of the aneurysm in the abdominal aortas and higher elastin/collagen ratio in the thoracic aortas, etc. The most probable reason for this discrepancy may be the wide variation of histology in the aneurysm tissues (Fig. 2). It has been reported that mechanical properties have close correlation with the histology in various tissues including atherosclerotic lesion (16) and bovine and porcine thoracic aortas (17, 18). The wide variation of the histology in the aneurysm tissues may cause the inconclusive results. We need to study the relation between the mechanical properties and histology of the aneurysm tissues.

Some limitations of our experimental methods require discussion. Perhaps foremost was that we evaluated mechanical properties with the initial elastic modulus representing elastic properties under small strain. We took this approach because more than 40% of the specimens broke before the strain in either direction reached 5%. However, stresses at failure $\sigma_\text{x}^\text{e}$ and $\sigma_\text{y}^\text{e}$ were much higher than the estimated in vivo stress $\sigma_\text{in vivo}$ (Table 1). Thus, the strain range used in the present study covers physiological state, although the maximum strain was relatively small compared to that of healthy arteries. We believe that the initial elastic moduli obtained in this study fairly represent the elastic properties of the aneurysm tissue in the physiological state.

Another limitation would be that we did not apply multiple deformations to the specimens because some of them were too fragile to withstand multiple loadings. Preconditioning is necessary for tissues obtained from healthy arteries. In case of severely diseased tissues, however, it might not be so important, because the effect of the preconditioning was minor for the specimens with advanced atherosclerosis (16). Multiple deformation protocols would be also necessary for accurate parameter estimation. We need to find a new specimen holding method to apply multiple deformations to fragile specimens. The strain applied to the specimen was not equibiaxial even though the specimens were stretched while the movement of the arms was set equal for both directions. It would be necessary to implement a feedback loop to control the strain or stress.

The specimens used in this study had been kept frozen and their mechanical properties were obtained at a room temperature. These indicate that smooth muscle activity had been completely lost. This might cause artifacts. To study the effects of the frozen storage and the ambient temperature, we measured the biaxial tensile properties of fresh and frozen porcine thoracic aortas at 37°C and at room temperature (23°C), and found that neither the initial elastic modulus nor the anisotropy index were affected significantly by the frozen storage and the ambient temperature (Fig. 6). Furthermore, it has been reported that the volume fraction of smooth muscle in the aortic aneurysm wall was almost 1/10 of the nonaneurysmal wall (7). Thus, we believe our room temperature measurements represent the mechanical response of the aneurysm wall to biaxial stretching.
Fig. 6  Comparison of stress-stretch ratio curves of fresh (a) and frozen (b) porcine thoracic aortas. Frozen specimens were kept at –18°C for 2 weeks. The method of the experiment was similar to that described in the materials and methods section except that the specimens were preconditioned and that mechanical properties were also measured at 37°C in an oxygenated Krebs-Henseleit solution.

In summary, we have reported biaxial mechanical properties of thoracic aneurysm tissues of various diseases. Our results show that aneurysm tissues were stiffer or more anisotropic than nonaneurysmal tissues in terms of their initial elastic modulus. We also indicated that the biaxial tensile properties couldn’t be estimated from the uniaxial test. Biaxial testing is important for the detailed analysis of mechanical properties of aneurysm wall.

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