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Submitted 24 September 2010; accepted 12 February 2011
Available online 25 March 2011

\textbf{KEYWORDS}
Intraluminal thrombus; Thrombus-covered wall; Biaxial mechanical test; Anisotropy; AAA rupture

\textbf{Abstract}  \textbf{Objective:} The intraluminal thrombus (ILT) present in the majority of abdominal aortic aneurysms (AAAs) plays an important role in aneurysm wall weakening. Studying the age-dependent elastic properties of the ILT and the thrombus-covered wall provides a better understanding of the potential effect of ILT on AAA remodelling.

\textbf{Materials and methods:} A total of 43 AAA samples (mean age $67 \pm 6$ years) including ILT and AAA wall was harvested. Biaxial extension tests on the three individual ILT layers and the thrombus-covered wall were performed. Histological investigations of the thrombi were performed to determine four different age phases, and to correlate with the change in the mechanical properties. A three-dimensional material model was fitted to the experimental data.

\textbf{Results:} The luminal layers of the ILT exhibit anisotropic stress responses, whereas the medial and the abluminal layers are isotropic materials. The stresses at failure in the equibiaxial protocol continuously decrease from the luminal to the abluminal side, whereby cracks, mainly oriented along the longitudinal direction, can be observed in the ruptured luminal layers. The thrombi in the third and fourth phases contribute to wall weakening and to an increase of the mechanical anisotropy of their covered walls. The material models for the thrombi and the thrombus-covered walls are in excellent agreement with the experimental data.

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doi:10.1016/j.ejvs.2011.02.017
Introduction

Aortic aneurysms are localised balloon-shaped expansions of the aorta. About 75% of aortic aneurysms are located in the abdomen. Most abdominal aortic aneurysms are asymptomatic, associated with smoking and high blood pressure and occur primarily in men over age 60 years. An untreated abdominal aortic aneurysm (AAA) is at high risk of rupture, which has an overall mortality rate of 90%. Several factors, including maximum AAA diameter, expansion rate of AAA, peak wall stress, and geometrical factors of the aneurysm, were explored to assess the likelihood of rupture. To date still no reliable criterion can predict the risk of AAA rupture in the final clinical decision. However, there is increasing evidence that patient-specific biomechanical factors may be more reliable in predicting AAA rupture than currently available clinical and biochemical parameters.

An intraluminal thrombus (ILT), present in the majority of AAAs, is a three-dimensional fibrin structure incorporated with blood proteins, blood cells, platelets and cellular debris. Many previous studies suggest that the presence of the ILT alters the wall stress distribution and reduces the peak wall stress in AAA. Thus, ILT serves as a mechanical cushion and is usually found at the rupture site of the AAAs. Vorp et al. proposed that the ILT also serves as a barrier to the oxygen supply from the lumen, possibly causing hypoxia of the aortic wall, and local ILT thickness might be a potential predictor of AAA wall strength.

To investigate the influence of ILT on the biomechanics of AAAs, it is pivotal to study and establish its biomechanical behaviours. Wang et al., for example, carried out uniaxial extension tests to evaluate the mechanical properties of the ILT, and three individual layers (i.e., luminal, medial and abluminal layers) were identified, suggesting that ILT is a heterogeneous, nonlinear elastic and isotropic material. Later biaxial extension tests showed that the luminal layer of the ILT behaves in an isotropic manner, which was consistent with the finding of previous uniaxial extension tests. Other recent mechanical studies focus on a variety of aspects regarding the ILT and correlation between the ILT and aneurysm wall weakening. However, the role of ILT in a rupture risk assessment of AAAs is still elusive and relevant data are lacking. Moreover, available experimental data show the biomechanical properties of the aneurysm wall; however, it is not clear whether or not the wall was covered with thrombus.

There are three aims of the present study. The first aim is to systematically investigate the biomechanical properties of the ILT and the AAA wall underneath the thrombus via biaxial extension tests. Such experimental data lead to a better and more comprehensive understanding of the complex structure of the ILT and its potential influence on growth and remodelling of AAAs. Based on these data, the development of more appropriate 3D material models, which are able to characterise the mechanical and growth (remodelling) behaviours of the ILT and the AAA wall, is then feasible, which is the second aim. The third aim is that we seek to identify the thrombus age by means of histology, which seems to be a first attempt within AAA research.

Materials and Methods

Material and specimen preparation

In total, 43 AAA samples (mean age 67 ± 6 years) including ILT and AAA wall were harvested from open surgical aneurysm repairs at the department of vascular surgery, Medical University Graz, Austria. This AAA material from human subjects was approved by the Ethics Committee. The maximum AAA diameters of these patients were mostly above 5.5 cm, which is the most commonly used criterion to assess increased risk of AAA rupture by clinicians. All samples, primarily from the anterior portion of the aneurysm, were stored in Dulbecco’s Modified Eagle’s Medium (DMEM) and tested within 6 h after retrieval. Square specimens, approximately 2 × 2 cm, were cut from the thickest part of the ILT sample and the corresponding AAA wall, where the local longitudinal direction was marked with a coloured thread sewn by surgeons. The mean thickness of each specimen was measured using the same method as by Sommer et al.

ILT heterogeneity has been reported and highlighted by several representative studies. In this study, for most ILT samples three individual layers (luminal, medial and abluminal layers) could easily be distinguished and peeled off (see Fig. 1). Four nylon sutures were fixed to each side of the square specimen with fish hooks, and four markers were placed at a distance of approximately 6 mm from each other at the centre of the specimen forming a diamond (see Fig. 2(a)).

Biaxial extension tests

Square specimens were mounted in a biaxial testing device via connecting four carriages by hooked nylon sutures (Fig. 2(b)) and were then submerged into a bath filled with DMEM and maintained at 37.0 ± 1.0 °C by a heater-circulation unit (Ecoline E 200; LAUDA, LAUDA-Königsfelden, Germany). The initial lengths between the two markers in each direction were also measured by a CCD camera before the start of the biaxial extension tests.

A stress-driven protocol was used during the testing procedure, where the engineering stresses (i.e., the first Piola-Kirchhoff stresses) served as a measure. The non-zero stresses have the form

\[ \sigma_{\text{engineering}} = \frac{f_i}{F_{\text{TX}}} \]

Conclusion: Our results suggest that thrombus age might be a potential predictor for the strength of the wall underneath the ILT and AAA rupture.
where $f_\theta$ and $f_L$ denote the measured forces in each direction, $T$ is the mean thickness in the unloaded configuration, and $X_\theta$ and $X_L$ are the initial dimensions of the square specimen along the circumferential and longitudinal directions, respectively, that is, 2 cm, (see Fig. 2). In this study the shear deformation was treated as negligible. Each specimen was tested using the following protocols:

- $P_{\theta\theta} : P_{LL} = 1:1.075 : 1.1 : 0.75, 0.5 : 1.1 : 0.25 : 1.1 : 0.25 : 1.1 : 0.25 : 1.1.$

For each protocol the ratio between the applied engineering stresses $P_{\theta\theta} : P_{LL}$ remained constant. We started with 15 kPa to do the biaxial protocol for the luminal, medial and abluminal layers, while the initially applied stress was determined to be 150 kPa for the degenerated AAA wall. Each time the load level increased by 5 kPa for the ILT and 50 kPa for the AAA wall, based on the previous stress values until mechanical ruptures occurred. Throughout the test, 2 kPa/s and 20 kPa/s were maintained during the loading cycle for the ILT and the AAA wall, respectively. The testing was performed with 10 loading and unloading cycles for each stress ratio, and the last (tenth) cycle was used for subsequent analysis.

**Data analysis**

The Cauchy stress $\sigma$ and stretch $\lambda$ were computed to quantify the biaxial biomechanical response of the tissue. With negligible shear components, the Cauchy stresses were determined as

$$
\sigma_{\theta\theta} = \frac{f_\theta}{tX_\theta}, \quad \sigma_{LL} = \frac{f_L}{tX_L}
$$

(2)

where $t$ is the mean thickness of the tissue in the current configuration, and $X_\theta$ and $X_L$ indicate the current dimensions of the square specimen along the longitudinal and circumferential directions, respectively. The assumption of
incompressibility then gives \( t_{X_{0}}X_{0} = TX_{0}X_{0} \) and the Cauchy stresses can be rewritten as
\[
\sigma_{\theta\theta} = \frac{f_{\theta\theta}}{T_{X_{0}}} \quad \sigma_{LL} = \frac{f_{LL}}{T_{X_{0}}}
\]  
(3)
where \( \lambda_{\theta} = x_{\theta}/X_{0} \) and \( \lambda_{L} = x_{L}/X_{0} \) represent the tissue stretches in each direction, which are measured at the markers.

**Biaxial mechanical evaluation**

For the equibiaxial stress-controlled protocol \((P_{w} : P_{L}=1 : 1)\), the mean peak stretch (MPS) values \((\lambda_{\theta, \text{max}}, \lambda_{L, \text{max}})\) for the three individual ILT layers and the thrombus-covered AAA walls in the circumferential and longitudinal directions were recorded and compared using paired \(t\)-test. The maximum tangential modulus (MTM) in the equibiaxial stress-controlled protocol was also calculated to assess stiffness at \( P_{w} : P_{L}=20\) kPa for the thrombus and at \( P_{w} : P_{L}=150\) kPa for the AAA wall. The applied engineering stress values, which caused the specimens to rupture for the equibiaxial protocol, were averaged for each tissue type. The cracks of the ruptured specimens after biaxial testing were studied to summarise some key features in this regard, specifically for the ILT. All values were statistically expressed as mean \(\pm SD\).

**Material model**

To model the mechanical characteristics of both ILT and thrombus-covered wall, we used a strain–energy function \(\psi\) which is based on a model developed for arterial walls,\(^{24,25}\) that is,
\[
\psi = \mu(\ell _{1} - 3) + \frac{k_{1}}{k_{2}} \left( \exp \left( k_{2} \left( (1 - \rho)(\ell _{1} - 3)^{2} + \rho(\ell _{4} - 1)^{2} \right) \right) - 1 \right),
\]  
(4)
where \(\mu > 0\) and \(k_{1} > 0\) are stress-like parameters with dimension (kPa), and \(\rho \in [0,1]\) and \(k_{2} > 0\) are dimensionless. By neglecting shear deformations, the invariants \(\ell _{1}\) and \(\ell _{4} > 1\) can be written as
\[
\ell _{1} = \lambda_{\theta}^{2} + \lambda_{L}^{2} + \lambda_{I}^{2}, \quad \ell _{4} = \lambda_{\theta}^{2} \cos^{2} \varphi + \lambda_{L}^{2} \sin^{2} \varphi
\]  
(5)
where \(\lambda_{\theta}, \lambda_{L}, \lambda_{I}\) in Eq. (5) are the principal stretches in the circumferential, longitudinal and radial directions, respectively. Both the ILT and the thrombus-covered wall samples are assumed to be incompressible, which requires that \(\lambda_{\theta} = \lambda_{L} = 1\). In Eq. (5), \(\varphi\) is a geometrical parameter that represents the angle between some fibre reinforcement and the circumferential direction; here, \(\varphi\) is treated as a phenomenological variable.

The Cauchy stresses in the circumferential and longitudinal directions are respectively \(\sigma_{\theta\theta} = \lambda_{\theta} \partial \psi / \partial \lambda_{\theta}\) and, \(\sigma_{LL} = \lambda_{L} \partial \psi / \partial \lambda_{L}\). Hence, with (5) and the incompressibility condition, we get\(^{23}\)
\[
\sigma_{\theta\theta} = 2(\lambda_{\theta}^{2} - \lambda_{\theta}^{2})^{2})\psi_{1} + 2\lambda_{\theta}^{2} \cos^{2} \varphi \psi_{4},
\]  
(6)
and
\[
\sigma_{LL} = 2(\lambda_{L}^{2} - \lambda_{L}^{2})^{2})\psi_{1} + 2\lambda_{L}^{2} \sin^{2} \varphi \psi_{4},
\]  
(7)
where the abbreviation \(\psi_{i} = \partial \psi / \partial \lambda_{i}, i = 1,4\), has been used. Note that the stress relations (6) and (7) are valid for zero shear deformation.

The related shear stress \(\sigma_{KL}\) then has the form \(\sigma_{KL} = \lambda_{\theta} \lambda_{L} \sin 2\varphi \psi_{4}\).\(^{23}\)

The data from the five biaxial protocols \((P_{w} : P_{L}=1: 0.75; 1: 1; 0.75; 0.5; 1: 1; 0.5)\) for each ILT and wall specimen associated with the circumferential and longitudinal directions were simultaneously fit to the material model (4) by using the optimization toolbox ‘Isqnonlin’ in Matlab 7.0. Consequently, the five parameters \((\mu, k_{1}, k_{2}, \varphi, \rho)\) were obtained. To measure the ‘goodness of fit,’ the square of the Pearson’s correlation coefficient was computed for both the circumferential and the longitudinal Cauchy stresses.

**Histology**

After mechanical testing the specimens were fixed in 10% neutral-buffered formalin (pH 7.4), embedded in paraffin using standard techniques and prepared for histological investigations.

Consecutive histological sections were cut at 4 \(\mu m\) and stained with Hematoxylin and Eosin (HE), Prussian blue (PB), Elastica van Gieson (EvG), Gömöri methenamine Silver stain (G) or Mallory–Cason Trichrome (also known as SFOG) to determine different specimen compositions. Subsequently, the sections were examined under a light microscope to study the thrombus compositions with regard to presence or absence of erythrocytes (SFOG), vital lymphocytes (HE) remaining from the blood stream, macrophages (HE), iron deposits (PB) from degraded erythrocytes, reticulin fibres (G), collagen fibres (G and EvG) and fibrin compositions (SFOG) with morphological differentiation between loose fibrin network or homogenised fibrin and other homogenised proteins. Statistical analysis was then performed to quantify the relative percentage of cellular contents for each specimen. We postulate a four-phase thrombus evolution within the AAA: phase I (very fresh), phase II (young), phase III (intermediate) and phase IV (old).

**Results**

In total, 33 luminal, 22 medial, 12 abluminal layers and 14 thrombus-covered walls were tested and analysed. The mean (outer) diameter of the AAA samples was 6.0 \(\pm 0.9\) cm \((n = 43)\) with a mean patient age of 67 \(\pm 6\) years. The mean measured thicknesses for the individual luminal, medial and abluminal layers were 2.53 \(\pm 0.45\) mm, 2.86 \(\pm 0.37\) mm and 2.15 \(\pm 0.29\) mm, respectively. The thickness of the AAA wall under the thrombus was 2.27 \(\pm 0.23\) mm. Highly degraded abluminal layers were often found to be very weak, greatly increasing the possibility of mechanical failure even at a very low load level. Clinical data of 36 patients are summarised in Table 1.

**Biaxial mechanical response**

**Intraluminal thrombus (ILT)**

The MPS and MTM values in the equibiaxial stress-controlled protocol \((P_{w} = P_{L} = 20\) kPa) for the luminal, medial,

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\(^{23}\) Tong et al. J. Tong et al.
abulminal layers and the fresh thrombi are summarised in Table 2. There is a clear indication of mechanical anisotropy for several luminal layers (n = 10), where the MPS \( \lambda_{\text{an}, \text{max}} \) and \( \lambda_{\text{L}, \text{max}} \) for the equibiaxial stress-driven protocol were 1.17 ± 0.03 and 1.09 ± 0.02, respectively (p = 0.0001). The MPS \( \lambda_{\text{L}, \text{max}} \) was found to be significantly smaller than for samples which behaved isotropically (p = 0.01), whereas there was no significant difference in

<table>
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<th>No.</th>
<th>Age</th>
<th>Gender</th>
<th>Max. D (cm)</th>
<th>Thromb. Age (phases)</th>
<th>Approx. time (from CT to OP)</th>
<th>TC (G/l)</th>
<th>CRP (mg/l)</th>
<th>FIB (mg/l)</th>
<th>Status</th>
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<td>M</td>
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<td>II</td>
<td>1 month</td>
<td>276</td>
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<td>(−)</td>
<td>&gt;1 month</td>
<td>140</td>
<td>5.8</td>
<td>379</td>
<td>a</td>
</tr>
</tbody>
</table>

Max. D, maximum diameter; Thromb. age in terms of phases I, II, III, IV, very fresh, young, intermediate, old thrombus; (−) means no thrombus in this patient or age determination failed; CT, X-ray computed tomography; OP, operation; (Rup) means ruptured aneurysm; TC, preoperative count of thrombocyte (normal value: 140–440 G/l); preoperative CRP, C-reactive protein (normal value: −8.0 mg/l); i and j denote values out of normal range; preoperative FIB, fibrinogen (normal value: 210–400 mg/l); s, symptomatic; a, asymptomatic status of patient.
Figure 3  Experimental data (symbols) with corresponding material model (solid curves) for (a)–(d) the anisotropic and isotropic luminal layers, (e) and (f) the medial layers, and (g) and (h) the abluminal layers of ILT specimens (patient 18) in the circumferential and longitudinal directions. The legend in plot (a) applies also to all others.
between both groups ($p = 0.25$). For the medial ($n = 22$) and abluminal ($n = 12$) layers, there was no significant difference in both MPS and MTM values in the circumferential and the longitudinal directions. Furthermore, the MPS values for the abluminal layers were found to be significantly larger than those of both luminal and medial layers at the same stress level (all $p$ values less than 0.01), suggesting a continuous increase in extensibility and decrease in stiffness from the luminal to the abluminal side in the biaxial mechanical behaviour of the ILT. The Cauchy stress-stretch plots in Fig. 3 show the biaxial data (symbols) of the different ILT tissues.

**Fresh thrombus**

In this study, four pieces of dark-red fresh thrombi harvested from four different patients ($66 \pm 3.6$ years, 4 males) were tested. The mean thickness was $6.87 \pm 0.51$ mm. Intriguingly, all which are not layer specific and homogeneous; they behaved isotropically under biaxial loading. Their biaxial mechanical behaviours were quite similar to the abluminal layers of old thrombi (see Fig. 3(g) and (h), and subsequent Fig. 6).

**Thrombus-covered AAA wall**

Our experimental results demonstrated that the thrombus-covered AAA walls ($n = 14$) exhibit an anisotropic exponential response associated with a larger circumferential stiffness, (see the symbols in Fig. 4). In the equibiaxial protocol ($P_{qq} = P_{LL} = 150$ kPa), the MPS and MTM values in the circumferential direction were significantly larger than those in the longitudinal direction, (see Table 3).

**Rupture stresses and cracks**

The engineering stresses that caused the specimens to rupture for the luminal, medial and abluminal layers were $60.5 \pm 6.2$, $41.2 \pm 6.5$ and $27.9 \pm 4.5$ kPa, respectively. For the thrombus-covered walls, the mean stress leading to the mechanical failure was $356.6 \pm 54.7$ kPa. Interestingly, cracks were more often found along the longitudinal direction at the centre of the specimens, for approximately 70% of the luminal layers in the phases II and III, (see Fig. 5). This type of crack was found to be independent of the hooks. However, there was no clear tendency of the cracks for the medial and abluminal layers, as well as the thrombus-covered walls, where ruptures were more likely initiated by the hooks, (see also Fig. 5).

**Material model**

The material model (4) was able to fit the three individual layers of the ILT very well, with a mean $R^2$ of $0.92 \pm 0.05$, $0.93 \pm 0.04$, $0.93 \pm 0.04$ and $0.94 \pm 0.03$ for the luminal, medial, abluminal layers and the fresh thrombus, respectively. The model results of the ILT are shown in Fig. 3 as solid curves, and the related parameters are summarised in Table 4.

The model (4) was also appropriate to characterise the biaxial mechanical responses of the thrombus-covered walls with a ‘goodness of fit’ $R^2$ of $0.93 \pm 0.04$. Fig. 4 shows the analytical results predicted by the model as solid curves, and the related parameters are summarised in Table 5. To find a correlation between thrombi and the thrombus-covered walls, the wall specimens were classified in two types: (1) walls covered by the younger thrombi (phase II) and (2) walls covered by the intermediate and old thrombi (phases III and IV; see also Table 5). The material model parameters for each group were then used to predict the mean stress-stretch responses of the ILT and the thrombus-covered wall, (see Figs. 6 and 7), respectively.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>MPS and MTM values (mean ± SD) in the equibiaxial stress-controlled protocol ($P_{qq} = P_{LL} = 150$ kPa) for the thrombus-covered (TC) wall ($n = 14$).</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC wall</td>
<td>$\lambda_{\text{max}}$</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
</tr>
<tr>
<td>1.07 ± 0.03</td>
<td>1.11 ± 0.04</td>
</tr>
</tbody>
</table>
cases, the aneurysm wall was also available for histological examination. EvG-stained sections of the thrombus-covered wall showed a complete loss of elastic fibres and myocytes, whereas relatively more collagen fibres were discerned in the media, (see Fig. 8(e)). Fig. 8(f) shows the morphological characteristics of thick and thin bundles of fibrin network. By comparing MTM values of all specimens at the same stress level of 20 kPa, it is found that the stiffness of the thrombotic material continuously increases with thrombus age for each individual ILT layer. Simultaneously, the luminal layer may become anisotropic during this ageing process, (see Fig. 10). A strongly positive correlation between the ILT thickness and the thrombus age is also observed, (see Fig. 11).

Discussion

In previous studies the ILT was identified as an isotropic and heterogeneous material by using both uniaxial and biaxial extension tests. For several luminal layers, we found, however, a clear indication of mechanical anisotropy. Hence, we conclude that the luminal layer of the ILT is, in general, not an isotropic tissue. We analysed that the stiffness of the anisotropic luminal layers in the longitudinal direction is (much) larger with respect to that in the circumferential direction or the luminal layers, which behaved isotropically, (see, e.g., Fig. 3). However, the circumferential stiffness at $P_{\text{de}}=P_{\text{LL}}=20$ kPa is not significantly different for both groups. Moreover, our experimental results suggest that both medial and abluminal layers are isotropic. To the authors’ knowledge no biaxial data are available in this regard prior to this study. The computed mean rupture stresses indicated that the stiffness of ILT tissues continuously decreases from the luminal to the abluminal layers.

Table 4  Model parameters (mean ± SD) for the anisotropic luminal (n = 10), isotropic luminal (n = 23), medial (n = 22), abluminal layers (n = 12) and the fresh thrombi (n = 4), see Eqs. (6) and (7).

<table>
<thead>
<tr>
<th>ILT</th>
<th>$\mu$ (kPa)</th>
<th>$k_1$ (kPa)</th>
<th>$k_2$ (–)</th>
<th>$\varphi$ (%)</th>
<th>$\rho$ (–)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal (Aniso)</td>
<td>9.7 ± 1.5</td>
<td>15.9 ± 4.3</td>
<td>2.7 ± 1.4</td>
<td>84.1 ± 10.7</td>
<td>0.33 ± 0.07</td>
<td>0.93 ± 0.04</td>
</tr>
<tr>
<td>Luminal (Iso)</td>
<td>8.2 ± 1.7</td>
<td>12.3 ± 3.7</td>
<td>0.6 ± 0.3</td>
<td>89.3 ± 2.3</td>
<td>0.03 ± 0.02</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>Medial</td>
<td>7.1 ± 1.9</td>
<td>6.0 ± 2.2</td>
<td>0.07 ± 0.1</td>
<td>86.7 ± 7.5</td>
<td>0.05 ± 0.05</td>
<td>0.93 ± 0.04</td>
</tr>
<tr>
<td>Abluminal</td>
<td>5.1 ± 1.1</td>
<td>2.9 ± 1.0</td>
<td>0.03 ± 0.01</td>
<td>89.1 ± 1.1</td>
<td>0.05 ± 0.01</td>
<td>0.94 ± 0.03</td>
</tr>
<tr>
<td>Fresh thrombus</td>
<td>4.9 ± 0.7</td>
<td>2.1 ± 0.4</td>
<td>0.02 ± 0.01</td>
<td>88.9 ± 0.3</td>
<td>0.04 ± 0.01</td>
<td>0.94 ± 0.02</td>
</tr>
</tbody>
</table>
abluminal side. It should be noted that the rupture stresses obtained in this study may not represent the stress level at which ILT rupture actually occurs. This is primarily due to the fact that a number of ruptures, specifically for the degenerated medial and abluminal layers, are initiated by the hooks on the edges, (see Fig. 5). About 70% of the cracks in the luminal layers in phases II and III were found along the longitudinal direction. This particular crack morphology is closely related to the pore orientations, shapes and protein bonds between two neighbouring pores within the fibrin networks, which prevail as a typical component in these two phases. In addition, our investigations suggest that fresh thrombi have very unique characteristics, which are, as stated previously, not layer specific and homogeneous, and which is in contrast with the old and more organized thrombi.

The importance of the aneurysm wall strength regarding AAA rupture assessment has been stressed by several prior studies.4,27 Our result is basically in agreement with an early study by Vande Geest et al.19 Compared to the normal aorta, the wall stiffening can be attributed to destruction of the long half-life elastin and failure of the collagen cross-linkage.30 In addition, by comparing the stress—stretch responses between the aneurysm walls covered with younger thrombi (phase II) and with intermediate and old thrombi (phases III and IV), our study verifies that there is an increase in the wall anisotropy when the thrombus gets older, (see Fig. 7). Thereby, a weakening of the wall occurs in the longitudinal direction, which may also imply that the older thrombi are related to aneurysm wall weakening.

As can be seen from Table 4, the model parameters \( \mu \), \( k_1 \) and \( k_2 \) continuously decrease from the luminal to the abluminal layer. In addition, both stiffness parameters \( \mu \) and \( k_1 \) are highest for the anisotropic luminal and lowest for the abluminal layers, respectively. There is no significant difference in each material parameter between the abluminal layer and the fresh thrombus (all \( p \) values are larger than 0.05). This indicates that in both (low and high) loading domains the stiffness of the ILT continuously decreases from the luminal to the abluminal side, whereas the biaxial mechanical response of the fresh thrombus is quite similar to the abluminal layer. For the luminal, medial and abluminal layers, the mean values of \( \varphi \) approach 90°; however, as previously stated, \( \varphi \) is here not treated as a fibre angle. As a measure of mechanical anisotropy, \( \rho \) is significantly larger for the anisotropic luminal layers than for the isotropic ILT layers as well as the fresh thrombi (all \( p \) values are less than 0.01).

In contrast with the ILT, the thrombus-covered wall has much higher values of the stiffness parameters \( k_1 \) and \( k_2 \), representing its significant load-bearing capacity in the high loading domain. Moreover, in regard to Table 5, a significantly smaller value of \( k_2 \) (responsible for the higher loading domain) and a larger value of \( \rho \) indicate that walls covered by an intermediate or old thrombus, type (ii), are much weaker in the high loading domain and more mechanically anisotropic when compared with walls covered by a young thrombus, type (i). Prior to the current study, several material models regarding the ILT and the

### Table 5 Model parameters (mean ± SD) for the thrombus-covered wall (n = 14).

<table>
<thead>
<tr>
<th>Wall type</th>
<th>( \mu ) (kPa)</th>
<th>( k_1 ) (kPa)</th>
<th>( k_2 ) (( e ))</th>
<th>( \varphi ) (°)</th>
<th>( \rho ) (°)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type (i)</td>
<td>6.0 ± 3.2</td>
<td>97.4 ± 26.6</td>
<td>173.7 ± 64.7</td>
<td>35.8 ± 6.0</td>
<td>0.25 ± 0.08</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>Type (ii)</td>
<td>7.1 ± 4.6</td>
<td>61.0 ± 35.4</td>
<td>114.7 ± 60.4</td>
<td>31.9 ± 4.1</td>
<td>0.32 ± 0.09</td>
<td>0.95 ± 0.02</td>
</tr>
</tbody>
</table>

Figure 6 Average stress—stretch model responses of anisotropic (AL) and isotropic (IL) luminal, medial (M), abluminal (A) layers and fresh thrombus (F) for the equibiaxial protocol using the mean model parameters of Table 4, where (l) and (h) denote circumferential (solid curves) and longitudinal (dashed curves) directions, respectively.

Figure 7 Circumferential and longitudinal mean stress—stretch responses of the thrombus-covered walls using the mean model parameters from Table 5. (l) and (h) denote circumferential and longitudinal directions; overall, Type (i) and Type (ii) denote all thrombus-covered walls, walls covered by the younger thrombi (phase II) and by the intermediate and old thrombi (phases III and IV), respectively.
AAA wall have been proposed. Nevertheless, some of these models were either derived from uniaxial extension tests or were based on 2D strain–energy functions, which are less appropriate to characterise the mechanical responses of 3D AAA tissues.

Data of thrombus age are very much lacking in the literature, especially for the ILT within AAAs. To further interpret the change in the mechanical properties, there is a need to classify all tested specimens within different age phases. All fresh thrombi belong to phase I and almost all isotropic luminal layers are of phase II; however, anisotropic luminal layers are either of phase III or IV. Medial layers cover phases II–IV, while abluminal layers belong mainly to phases III and IV. For each individual ILT sample, the luminal and medial layers usually have the same age phase. Initially, the thrombotic materials are very compliant and isotropic (phase I). Starting from phase II, they become stiffer due to the formation of the loose fibrin network. In this situation, the thrombi are sponge-like materials with fluid inside and they are mechanically isotropic. During phase III, the thrombi become much stiffer as the fibrin networks are composed of much thicker bundles. The thrombotic material can be either isotropic or anisotropic for different individual ILT layers. As a consequence of the fibrin network disruption and more condensed residue proteins, the thrombi are much stiffer in

Figure 8 Representative histological images characterizing the morphology of the thrombotic material in four age phases: (a) very fresh, (b) young, (c) intermediate and (d) old. Original magnification 10×. Histological images of (e) the thrombus-covered wall consisting of three individual layers (original magnification 4×) and (f) the fibrin network mixed with both thick and thin bundles highlighting their distinct morphological characteristics (original magnification 40×).
phase IV, comprising the anisotropic luminal and the isotropic medial and abluminal layers.

One possible explanation for the mechanical isotropy of the luminal and medial layers in the phases II and III is that the fibrin network plays a dominant role in the biaxial stretching as compared to erythrocytes or other degraded proteins during the younger phases. Importantly, fibrin polymers have a large extensibility and elasticity when compared with other protein polymers.33,34 Factors to trigger the mechanical anisotropy of several luminal layers in the phases III and IV can be summarised as follows. First, differences between the thick and thin bundles within the fibrin network, evident from histology, may lead to different mechanical properties during biaxial stretching. The microstructure might be a factor for the mechanical anisotropy of the thrombotic material, which needs to be further investigated. Second, as a scaffold, pore densities, shapes and orientations of the fibrin network in the younger phase may considerably influence the distributions and depositions of residue small proteins in the later phases. Thus, the older thrombotic material may exhibit mechanical anisotropy probably due to unequal distributions of residue proteins. Finally, shear stresses due to the blood stream should also be, to some extent, taken into account in such a complex and potentially dynamic environment.

All laboratory data given were sampled $4\text{e}48$ h prior to surgery. All patients suffered from hypertension, whereas diabetes were only found in 7 (19%) of 36 patients. In 30 (83%) patients, aneurysms are asymptomatic. Smoking statistics show that 85% of the patients are active smokers or smoked previously. Further, we roughly estimated the thrombus age for each patient by integrating age information of the three individual ILT layers, (see Table 1). Note, however, that a few specimens with success in mechanical testing failed to determine the thrombus age. Approximate time from preoperative X-ray computed tomography (CT) to operation (OP) for the patients whose thrombi are in the phases III and IV (these are 12 patients) are equal or less than 1 week (3.7 ± 2.1 days, mean ± SD) except for patient 21 (1 month). In 10 (80%) of these 12 patients, maximum diameters are equal to or larger than 6 cm. By contrast, patients with a younger thrombus (phase II), which are 17, hold a much longer time from CT to OP, at least 1 month for 14 patients (82%) out of these 17.

As an inflammatory indicator, preoperative CRP values are outside the normal range for 7 (60%) of 12 patients whose thrombi are in the phases III and IV. Meanwhile, 70% of these 12 patients have larger values of preoperative fibrinogen over the normal range. It is well known that fibrinogen is a critical index to evaluate the fibrin content of the thrombi, since it is essential for coagulation of blood and fibrin formation.35 Preoperative values of thrombocyte count for most patients (91%) are within the normal range. Moreover, patient 18 is the only one whose aneurysm ruptured before surgery. It should also be noted that this patient is the only one who showed three different types of ILT’s, including fresh, young and old thrombi (phases I, II and IV). This indicates that the ILT can be a complex structural material with different biomechanical properties even in the same AAA sample.
Peak wall stress and wall strength are two major potential predictors of AAA rupture. In fact, previous studies have substantiated that the ILT plays an important role in the AAA wall weakening. Therefore, more investigative attention should be given to the wall strength underneath the ILT, which is of crucial interest to the assessment of AAA rupture. Vande Geest et al. first developed a noninvasive technique to evaluate AAA wall strength in vivo. The present study suggests that the thrombus age is a critical factor to examine the strength of its covered wall. Consequently, the thrombus age, combined with other key predictor variables such as maximum diameter, peak wall stress and wall strength, may give a more comprehensive consideration on AAA rupture assessment. The corresponding mathematical model, if developed, would be a promising technique to link patientspecific ILT properties to the AAA wall strength.

There are also some limitations in the present work. The proposed age determination of thrombus does not provide the actual number of days for the specimens but rather the relative age phases. The main reason is that there is no cellular reaction (inflammatory response) for the ILT to organise the thrombotic material, which differs from thrombi in small arteries and veins. A full transversal section of the thrombus from the lumen to the ablumun, however, may be helpful to explore more morphological characteristics in histology and to further analyse the microstructure of ILT with thrombus ageing. Although biaxial extension tests can essentially characterise the biaxial mechanical behaviours of both ILTs and wall tissues, the ultimate tensile strength for both tissue types cannot be measured by carrying out this experimental method. It would also be good to compare the failure properties of ILTs with different ages and the walls that they cover.

Conclusion

The main finding of this article is that the luminal layer of the ILT may not be an isotropic material. Compared to isotropic luminal layers, anisotropic samples have remarkably larger longitudinal stiffnesses and are frequently associated with highly degraded medial and abluminal layers. Moreover, it appears that the present biaxial study is the first one which documents that the mechanical behaviours of fresh thrombi are quite similar to those of the abluminal layers in old (laminated) thrombi. Another key finding in this study is that we may determine the relative age phases of the thrombi, which are critically important and novel in AAA research. Further investigations show that the mechanical properties of the thrombotic material may change significantly with the thrombus age, and, remarkably, the older thrombi are related to aneurysm wall weakening. These findings provide us with an additional perspective into the initiation and progression of the ILT in the biomechanical AAA environment. It is, therefore, concluded that the thrombus age is a potential predictor for the strength of the wall underneath the ILT as well as AAA rupture.

Conflict of interest

None.

Acknowledgements

We acknowledge Dr. Gerhard Sommer from the Institute of Biomechanics at Graz University of Technology for his help during the experimental tests.

References


