Research Paper

Human thoracic and abdominal aortic aneurysmal tissues: Damage experiments, statistical analysis and constitutive modeling

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\textbf{A B S T R A C T}

Development of aortic aneurysms includes significant morphological changes within the tissue: collagen content increases, elastin content reduces and smooth muscle cells degenerate. We seek to quantify the impact of these changes on the passive mechanical response of aneurysms in the supra-physiological loading range via mechanical testing and constitutive modeling. We perform uniaxial extension tests on circumferentially and axially oriented strips from five thoracic (65.6 years $\pm$ 13.4, mean $\pm$ SD) and eight abdominal (63.9 years $\pm$ 11.4) aortic fusiform aneurysms to investigate both continuous and discontinuous softening during supra-physiological loading. We determine the significance of the differences between the fitted model parameters: diseased thoracic versus abdominal tissues, and healthy \textit{(Weisbecker et al., J. Mech. Behav. Biomed. Mater. 12, 93–106, 2012)} versus diseased tissues. We also test correlations among these parameters and age, Body Mass Index (BMI) and preoperative aneurysm diameter, and investigate histological cuts. Tissue response is anisotropic for all tests and the anisotropic pseudo-elastic damage model fits the data well for both primary loading and discontinuous softening which we interpret as damage. We found statistically relevant differences between model parameters fitted to diseased thoracic versus abdominal tissues, as well as between those fitted to healthy versus diseased tissues. Only BMI correlated with fitted model parameters in abdominal aortic aneurysmal tissues.

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1. Introduction

Cardiovascular diseases, one of the leading causes of death in the Western world, account for 40% of the deaths in the European Union (Nichols et al., 2012). Among these diseases, aortic aneurysms stand out for their high mortality rate in cases of rupture; 85–95% for abdominal aortic aneurysms (AAA) (Kniemeyer et al., 2000). Aortic aneurysms are permanent dilations of the vessel exhibiting a diameter 50% larger than that in health, or for AAA specifically, a diameter larger than 3 cm (Shimizu et al., 2006). Currently, the only treatment for an aortic aneurysm, which may grow and rupture, is elective surgical repair (Humphrey and Holzapfel, 2012). Such surgical repairs are either open or endovascular (catheter-based) procedures, where the latter generally reduces pain and hospitalization time for patients. Unfortunately, the surgeries themselves carry high risk and do not necessarily increase the survival rate (Lederle et al., 2002). Hence there is a great need to more accurately assess both AAA rupture risk and the risk of specific surgical procedures to aid decision making in the clinical setting.

Different clinical approaches do exist to estimate the rupture risk of aneurysms. Most modern hospitals use the diameter criterion which recommends aneurysm repair if the diameter exceeds 5–5.5 cm. Despite its rudimentary nature this criterion can be measured with standard devices, such as ultrasound, and the result is immediately available. Another often used criterion is the growth rate of the aneurysm diameter. To effectively measure the growth rate of an aneurysm it must be detected early such that time is available for monitoring and decision making. While both diameter and growth rate initially appear relatively easy to measure, variations in imaging planes and the non-circular nature of most aneurysms can lead to significant variations in the results. To overcome these disadvantages Kitagawa et al. (2013) suggested to measure the volume of an aneurysm using precise 3-D imaging methods in conjunction with modern image segmenting software, but this has not yet been applied clinically. Martin et al. (2013) estimated the rupture potential of ascending thoracic aneurysms by calculating a failure diameter, the diameter when tension within the wall was higher than the ultimate tension, using the law of Laplace in combination with data from uniaxial mechanical tests. Despite gross simplifications, results compared well with those observed clinically. There is still significant room for improvement of rupture-risk prediction methods for aneurysms, see, e.g., Vorp (2007) or Humphrey and Holzapfel (2012), or Raut et al. (2013) which describes the influence of geometric and biomechanical factors on risk estimations.

Clinical acquisition of patient-specific 3-D images opens the possibility to estimate more sophisticated rupture-risk predictors based on finite element (FE) analysis and tissue biomechanics. Accurate constitutive models for the tissue components are crucial in generating accurate simulation results. Biomechanicians generally model healthy arterial wall as an isotropic elastin matrix with reinforcing collagen fibers which are symmetrically oriented and either well-aligned (Holzapfel et al., 2000) or dispersed Gasser et al. (2006) in orientation. Contrary to healthy aortic tissues, little experimental data on the stress-strain response of aneurysmal tissues currently exist for use in calibrating FE simulations of AAA tissues. Vande Geest et al. (2006) performed biaxial extension tests on AAA samples and clearly showed nonlinear, anisotropic responses which were stiffer in the circumferential direction. Tong et al. (2011) reported similar anisotropic responses for AAA wall tissues covered by intraluminal thrombus (ILT). Sokolis et al. (2012) performed uniaxial extension tests to failure on separated layers of ascending thoracic aortic aneurysms (TAAs) and also reported that the tissue was stiffer in the circumferential direction.

There are other constituents, beyond the vessel wall, present in aortic aneurysms, constituents which potentially impact the overall mechanical behavior. ILT often covers the inner vessel walls of AAAs and should be considered in accurate FE models of aneurysms. Tong et al. (2011) also tested the three individual layers (luminal, medial and abluminal) of ILTs in biaxial extension and reported that the luminal layer showed an age-dependent mechanical response, starting as isotropic and turning anisotropic with age, in contrast the medial and abluminal layers were always isotropic. In contrast, both Wang et al. (2001) and O’Leary et al. (2014a) evaluated the biomechanics of ILT and concluded that the mechanical behavior was isotropic for all layers of the ILT. In a subsequent work Tong et al. (2014) quantified changes in the dissection energy of ILT with age, considering four stages of development, and connected these data to structural remodeling the tissue. Plaques – containing lipids, fibro-fatty composites and calcifications – constitute another structural element within vessel walls (Wu et al., 2013). Chai et al. (2013) performed indentation tests on plaque samples with low calcium content and showed a large variation in the stiffness within individual plaques. Mulvihill et al. (2013) further demonstrated that different plaques show significant variations in mechanical response and they identified voids within the plaques which increased the vulnerability of the whole structure.

In light of the potential for supra-physiological loading, researchers have extended constitutive models for vascular tissues to include effects from damage, capturing both continuous and discontinuous softening. In continuous theory softening (damage) accumulates throughout the entire load history while in discontinuous theory damage accumulates only when the applied loads exceed the previous maximum in the loading history. Balzani et al. (2006) introduced a model for discontinuous damage in the collagen fibers of the tissue and subsequently calibrated it successfully using one experimental data set from a human carotid artery (Balzani et al., 2012). Rodríguez et al. (2006) proposed another approach where collagen fibers were modeled as worm-like chains, and where the discontinuous softening was realized by considering individual strain energies for individual collagen fiber bundles and a beta-distribution of the rupture strain until fracture. Subsequently, Rodríguez et al. (2008) implemented this approach in an FE code to estimate the rupture risk of AAA tissues. Peña et al. (2009) developed a damage model for arterial tissues that accounts for both continuous and discontinuous softening, and that considers different damage evolutions for the matrix and two distinct fiber families. Using a complex experimental plan to determine the necessary parameters the authors fit both the
preconditioning behavior and the Mullins’ type softening of arterial tissues accurately. Gasser (2011) developed a microfiber model to describe the failure of collagen fibers due to irreversible rearrangements and loss of proteoglycan bridges which cause softening during supra-physiological loading. Using material parameters estimated from the macrosopic mechanical tests, the model captures preconditioning of the tissue as well as both the low and high-strain regions of the tissue’s stress-strain response. Rickaby and Scott (2013) modified a transversely isotropic eight-chain model by adding softening functions for the primary loading path, the unloading and reloading paths. The authors fitted longitudinal and circumferential data from a human thoracic aorta with excellent accordance, but unfortunately the model uses numerous parameters with unclear physical meanings. Recently, Schmidt et al. (2014) proposed a statistical approach to describe microscopic damage evolution in soft collagenous tissues. Within that model the statistical distributions of proteoglycan orientation, fibril length parameters and ultimate proteoglycan stretch were also considered. The applicability of the model approach within a FE framework was shown by simulating the overexpansion of simplified atherosclerotic arteries.

Efforts have been made to study specific surgical procedures which may generate high stresses and damage in the vasculature. Researchers have applied FE simulations to study specific medical applications, e.g., balloon-angioplasty (Balzani et al., 2006), stent interactions with vasculature (Mortier et al., 2010) and arterial clamping (Famaey et al., 2012). Vande Geest et al. (2008) applied FE analysis to estimate rupture-risk of patient’s AAAs using only the estimated maximum principal stresses in the aneurysm wall. The authors tested simulations including either isotropic or anisotropic material models and while neither was able to significantly discriminate ruptured versus non-ruptured AAAs, the authors suggest, based on statistical analysis, that anisotropic models of patient-specific AAAs may help better identify AAAs at high risk. Badel et al. (2014) calculated the dissection risk of a coronary artery during the early stages of balloon angioplasty using FE analysis which considered the presence of a fibrous plaque and the medial layer imbedded in myocardium and epicardium. These simulations, using cohesive interfaces to model damage, showed a high risk of plaque detachment at the shoulder region of the plaque (in accordance with clinical findings) and a second risk zone within the arterial media where damage initiated at a very early stage of the procedure. Iannaccone et al. (2014) simulated, using the damage model of Balzani et al. (2006), the influence of lumen geometry, plaque morphology and composition on damage initiation and lumen gain during balloon angioplasty. The authors showed that eccentricity of the plaque causes high stresses in uncovered areas, stress which could lead to an increased failure rate during the procedure. Balakhovsky et al. (2014) estimated the damage evolution and failure risk in growing aneurysms using an approach developed by Volokh and Vorp (2008). The authors calculated the rupture potential of two basic geometries, one representing saccular aneurysms and the other fusiform aneurysms, with different initial fiber properties and found a range of parameters that lead to aneurysm rupture. While they reach qualitative agreement with clinical observations, they have not validated their estimations with model geometries and mechanical properties derived from human aneurysmal tissues. Romo et al. (2014) determined regions of higher rupture risk by calculating the stress distributions and thickness evolution of the vessel wall during inflation tests on ascending TAA tissues. Contrary to the generally accepted theory that the tissues rupture at the location of the highest stress the authors claimed that locations of greatest reduction in thickness are most likely to fail.

The development and the growth of an aortic aneurysm include significant morphological changes within the vessel wall – collagen content increases, elastin content reduces and smooth muscle cells degenerate (see, e.g., Tsamis et al., 2013) – and little experimental data exist for the stress-strain response of aortic aneurysms in the supra-physiological loading range. In this study we seek to quantify the impact of these changes on the passive mechanical response of diseased aortic tissues by estimating appropriate material parameters via mechanical testing and constitutive modeling. In our previous work, we modeled the supra-physiological stress-stretch response of the healthy thoracic and abdominal aortic tissues with an anisotropic pseudo-elastic damage model (Weisbecker et al., 2012). To facilitate direct comparison we assume that this model is also valid for diseased, i.e., remodeled, tissues. To this end we perform uniaxial extension tests on circumferentially and axially oriented strips from six thoracic and eight abdominal aortic fusiform aneurysms. By repeating three loading cycles per loading level we investigate both continuous and discontinuous softening. We fit the resulting data with our previous pseudo-elastic constitutive model to quantify damage that accumulates in the aortic tissue causing softening. We use the Wilcoxon rank sum test to determine the significance of differences between the fitted material parameters: diseased thoracic versus abdominal tissues, and healthy (from previous) versus diseased tissues. We also test correlations of our parameters with age, Body Mass Index (BMI) and preoperative aneurysm diameter. To gather additional information to interpret model parameters, we investigate histological cuts following standard procedures.

2. Materials and methods

2.1. Constitutive model for arterial tissue including damage

Following Weisbecker et al. (2012) to model the material response of aortic tissues during supra-physiological loading, and to facilitate comparison between healthy and diseased tissues, we postulate a decoupled representation of a strain-energy function $\Psi$ and use a similarly decomposed pseudo-elastic damage model as (Ogden and Rolixirh, 1999)

$$\Psi(j, C, n_{ij}) = \Psi_{\text{vol}}(j) + \Psi^{\text{vol}}(C) + \sum_{i=1}^{4,6} [n_{ij} \Phi_{ij}(C) + \Phi_{ij}(n_{ij})], \quad i = 4, 6 \tag{1}$$

where here, and in all that follows, the index $i=4,6$ does not employ Einstein summation notation, instead it refers to a specific family of collagen fibers, $\Psi_{\text{vol}}$ is a strictly convex
function which describes the volumetric elastic response, $\Psi_m^0$ describes the isochoric strain energy of the undamaged matrix material (the superscript 0 denotes an undamaged material), $\Psi_{f,i}^0$ describes the isochoric strain energy of the undamaged fiber families, and $\eta_{f,i}$ denote (smooth) damage functions of the two fiber families (N.B. damage only accumulates in the deviatoric part of the strain-energy function and the material remains isochoric even under induced damage). Furthermore, $J = \det F > 0$ is the determinant of the deformation gradient $F$, $\mathbf{C} = F^T \mathbf{F}$ is the modified right Cauchy–Green tensor with $F = (J^{-1/3}) \mathbf{I}$ (Holzapfel, 2000), and $\eta_{f,i} \in [0, 1]$ denote damage variables of the two fiber families defined as (Weisbecker et al., 2012)

$$\eta_{f,i} = 1 - \frac{1}{r_i} \text{erf} \left( \frac{1}{m_i} \left( \Psi_{f,i}^{\text{max}} - \Psi_{f,i}^0 \right) \right),$$

where $\Psi_{f,i}^{\text{max}}$ denote the maximum strain energies obtained in the fiber families over the deformation history, $r_i > 1$ characterizes the maximum damage that can be induced in a fiber family under loading, and $m_i > 0$ determines how fast softening accumulates in a fiber family (N.B. we assume that damage evolves similarly in both fiber families so that damage can be fully described by the two parameters $r_i$ and $m_i$). Small values of $m_i$ indicate that significant damage occurs at relatively small strains, whereas larger values of $m_i$ indicate that damage will progress more slowly. We characterize damage induced in the fiber families by the minimum of the damage variables as

$$\eta_{f,i}^{\text{min}} = 1 - \frac{1}{r_i} \text{erf} \left( \frac{1}{m_i} \Psi_{f,i}^{\text{max}} \right).$$

Following Holzapfel et al. (2000) and Gasser et al. (2006) we model the arterial wall as a composite of matrix elastin with two dispersed, reinforcing collagen fiber families. We assume the non-collagenous matrix is isotropic and model it using the neo-Hookean strain-energy function

$$\Psi_m^0 = \frac{\mu}{2} (I_1 - 3),$$

where $I_1 = \text{tr} \mathbf{C}$ is the first invariant of $\mathbf{C}$ and $\mu > 0$ corresponds to the shear modulus of the material in the reference configuration. Based on experimental justification from Schriefl et al. (2012b) we assume two reinforcing collagen fiber families and model them using the anisotropic strain-energy functions:

$$\Psi_{f,i}^0 = \frac{1}{2 \kappa_i} \left[ \delta_{ij} \epsilon_i \epsilon_j - 1 \right] - 1,$$

where the pseudo-invariants $I_1^i = \mathbf{C} \cdot \mathbf{M} \otimes \mathbf{M}$ and $I_2^i = \mathbf{C} \cdot \mathbf{M} \otimes \mathbf{M}$ correspond to the squares of the stretches of the fibers in the fiber directions. The vectors $\mathbf{M}$ and $\mathbf{M}$ denote the mean directions of the collagen fibers in the reference configuration. Since the fibers are located in the tangential plane of the artery and symmetrically oriented with respect to the circumferential direction of the artery both $\mathbf{M}$ and $\mathbf{M}$ are uniquely defined by the structural parameter $\varphi$, which defines the angle between the circumferential direction of the artery and the fiber directions; in the present study the parameter $\varphi$ was used as a phenomenological (material) parameter. The dispersion parameter $\kappa \in [0, 1/3]$ describes the radial symmetry of dispersion of the fiber orientations about the mean fiber directions, $k_1$ is a stress-like parameter and $k_2$ is a dimensionless parameter. Herein the anisotropic strain-energy functions (5) only contribute to $\Psi$ when the fibers are extended (and not in compression), strictly when either $l_i > 1$ and/or $l_i > 1$. If one or more of these conditions is not satisfied then we omit the corresponding contributions of (5).

With (1)–(5) we calculate the second Piola–Kirchhoff stress tensor as $\mathbf{S} = 2 \Psi / \Omega = \mathbf{S}_0 + \mathbf{S}_1$, where $\mathbf{S}_0 = 2 \Psi^{\text{vol}} / \Omega$ and $\mathbf{S}_1$ is the isochoric second Piola–Kirchhoff stress tensor which we calculate as

$$\mathbf{S} = \mathbf{S}_0 + \sum_{i=4,6} \eta_{f,i}^0 \mathbf{S}_{f,i}^0 / \Omega,$$

where $\mathbf{S}_{f,i}^0$ and $\mathbf{S}_{f,i}^0$ are isochoric second Piola–Kirchhoff stress tensors according to the primary loading curve. With a standard push-forward operation we obtain the Cauchy stress tensor $\sigma = J^{-1} F S F$. For a more detailed discussion of the constitutive model the reader is referred to Weisbecker et al. (2012) and references therein.

### 2.2. Experimental protocol

#### 2.2.1. Sample preparation

We harvested 12 abdominal (69.7 years ± 4.4, mean ± SD) and seven thoracic (64.5 years ± 12.3) tissue samples from true fusiform aortic aneurysms undergoing open surgical repair at Universitäre Ziekenhuizen (UZ) Leuven, Belgium. We stored all samples in physiological solution and froze them for transportation to Graz University of Technology, Austria, where they were stored at −20 °C until experimental testing.

After unfreezing each sample, we carefully removed loose connective tissue attached to the adventitia so that only the wall itself remained for mechanical testing. With a punching tool we cut out rectangular samples, measuring 4 mm in width and at least 15 mm in length, in both the axial and the circumferential directions of the tissue. We measured sample thickness with a videotelemeter at four different points distributed evenly on each sample. To get a representative thickness for each sample we calculated the mean value of the four measurements. Finally, we stored the samples in physiological solution between preparation and testing to prevent dehydration.

In light of the available quality (some highly calcified and size (some too small) of the samples, we were able to test thoracic tissues from six patients (denoted as T.1–T.6) and abdominal tissues from eight patients (A.1–A.8). To increase the amount of mechanical data we took more than one sample pair per patient (denoted with an additional index, e.g., A.8.1), if the size of the original tissue sample was sufficient. Table 1 shows the available patient-specific data for the patients where we list only five out of six total patients with TAAs because for one patient we had no data.

Unfortunately we lost the data sheet (including sample-specific information) for one thoracic sample during transportation from Leuven, Belgium, to Graz, Austria, and thus we calculate the given mean value from the remaining six samples.

The Ethical Committee of UZ Leuven approved the use of material from human subjects for the present study (approval number B32220071899).
Table 1 – Patient-specific data: ‘M’ = male; ‘F’ = female; ‘BMI’ = Body Mass Index; ‘Diameter’ = patient’s maximum pre-operative aneurysm diameter; ‘NA’ = data not available.

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Gender (M/F)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>BMI</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.1</td>
<td>F</td>
<td>75</td>
<td>158</td>
<td>45</td>
<td>18.02</td>
<td>46</td>
</tr>
<tr>
<td>T.2</td>
<td>F</td>
<td>78</td>
<td>167</td>
<td>70</td>
<td>25.09</td>
<td>58</td>
</tr>
<tr>
<td>T.3</td>
<td>F</td>
<td>73</td>
<td>156</td>
<td>53</td>
<td>21.77</td>
<td>NA</td>
</tr>
<tr>
<td>T.4</td>
<td>M</td>
<td>51</td>
<td>173</td>
<td>83</td>
<td>27.73</td>
<td>48</td>
</tr>
<tr>
<td>T.5</td>
<td>M</td>
<td>51</td>
<td>186</td>
<td>98</td>
<td>28.32</td>
<td>49</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.1</td>
<td>M</td>
<td>73</td>
<td>175</td>
<td>85</td>
<td>27.75</td>
<td>73</td>
</tr>
<tr>
<td>A.2</td>
<td>M</td>
<td>64</td>
<td>178</td>
<td>73</td>
<td>23.04</td>
<td>59</td>
</tr>
<tr>
<td>A.3</td>
<td>M</td>
<td>74</td>
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<td>83</td>
<td>31.62</td>
<td>60</td>
</tr>
<tr>
<td>A.4</td>
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<td>71</td>
<td>176</td>
<td>88</td>
<td>28.40</td>
<td>70</td>
</tr>
<tr>
<td>A.5</td>
<td>F</td>
<td>70</td>
<td>169</td>
<td>75</td>
<td>26.20</td>
<td>60</td>
</tr>
<tr>
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<td>F</td>
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<td>165</td>
<td>87</td>
<td>31.95</td>
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</tr>
<tr>
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<td>M</td>
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<td>75</td>
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</tr>
<tr>
<td>A.8</td>
<td>M</td>
<td>74</td>
<td>166</td>
<td>70</td>
<td>25.40</td>
<td>85</td>
</tr>
</tbody>
</table>

We provide additional information on the medical background and lifestyle of the patients in Table A1 of Appendix A.

2.2.2. Uniaxial tension test

We performed uniaxial mechanical tests on circumferentially and axially oriented strips from five thoracic (65.6 years ± 13.4, mean ± SD) and eight abdominal (63.9 years ± 11.4) aortic fusiform aneurysms. The tests were performed within a bath of physiological solution, kept at a constant temperature of 37°C, using a uniaxial testing machine. To ensure a secure connection to the testing device clamps and to prevent slippage we glued sandpaper to the ends of the samples. We also attached small rectangular foam markers to the samples in order to measure the displacement during testing using a videoextensimeter. Our testing procedure is force driven, where we calculated forces which result in the desired first Piola–Kirchhoff stresses of 25, 50, 75, 100, 200 and 300 kPa. For each load step we performed three load cycles in order to observe both discontinuous softening (in the first loading cycle) and continuous softening (in the two following cycles to the same maximum load). To avoid dynamic or viscoelastic effects we performed testing under quasi-static conditions with a displacement rate of 5 mm/min.

2.2.3. Data analysis and model fit

We completed all data analyses using a semi-automatic fitting procedure implemented in Matlab (R2011b, The MathWorks, Inc., Massachusetts, USA). To start, we preprocessed the force–displacement data sets to eliminate those data recorded either after rupture of the sample or when the sample slipped out of the testing clamps. In order to fit our constitutive damage model to the experimental data, we next calculated the stretch \( \lambda = l/L \), where \( l \) is the deformed length of the sample (related to the displacement of the sample) and \( L \) is the reference length of the undeformed sample, both in the direction of loading. Subsequently we calculated the Cauchy stress \( \sigma \) as

\[
\sigma = \frac{f}{TW} \lambda, \tag{7}
\]

where \( f \) is the measured force, \( T \) is the initial thickness and \( W \) is the initial width of the material, cf. Weisbecker et al. (2012). To reduce noise, we filtered the experimentally determined stretch and stress data with a Savitzky–Golay Filter of order two. We varied the frame size of the filter for each specimen (minimum of 41 data points to maximum of 151) depending on the noise but kept this as low as possible to avoid manipulating the data.

Given the experimentally determined stretch–stress data for each sample, we used the nonlinear least-square trust region algorithm implemented in Matlab to minimize the objective function

\[
\chi = \sum_{i=1}^{n} [\sigma_{i}^{\text{exp}} - \sigma_{i}^{\text{mod}}(\mathbf{x})]^2, \quad j \in \{\text{mat, dam}\}, \tag{8}
\]

where \( n \) is the number of data points considered, \( \sigma_{i}^{\text{exp}} \) are the Cauchy stresses determined experimentally and \( \sigma_{i}^{\text{mod}} \) are the corresponding values predicted by our constitutive model including pseudo-elastic damage (1)–(6) for both axial and circumferential directions simultaneously, and \( \mathbf{x} \) denotes a vector of model parameters (the subscript \( j \) denotes which of the parameters are fit with \( j = \text{mat} \) referring to material parameters and \( j = \text{dam} \) referring to damage parameters). To calculate the Cauchy stress response of the model we determined the lateral and the through-the-thickness stretches by invoking the incompressibility condition and an additional constraint that the stress in the lateral direction equals zero. Specifically, we first fitted the material parameters \( \mathbf{x}_{\text{mat}} = [\mu, k_1, k_2, \phi, \kappa] \) to the primary loading curve. To improve the quality of our fit in the toe region of the loading curve, we performed an initial fit in this toe region separately to obtain the shear modulus \( \mu \) which is largely responsible for the material behavior at low deformations. Next we obtained the damage parameters \( \mathbf{x}_{\text{dam}} = [\mathbf{r}_t, \mathbf{r}_l] \) by repeating the fitting procedure, i.e., by minimizing (8) using the fitted material parameters and the entire loading history. Note that we tested different sets of initial guesses for the model parameters as these may lead to different fitting results (Ogden et al., 2004), yet all physically reasonable initial guesses resulted in nearly identical model parameters. We reported the complete set of fitted model parameters (material and damage) as well as the
median values with their corresponding interquartile ranges, and provide the coefficient of determination $R^2$ to evaluate the goodness of fit.

2.2.4. Statistical analysis
We used statistical methods to determine if our fitted model parameters correlate significantly with a patient’s age, BMI and preoperative aneurysm diameter using the rank correlation coefficient $r_c$. We also tested the significance of any correlations by calculating the confidence interval with the Fisher transformation. Due to the low number of thoracic samples we performed these calculations only for the abdominal aortic samples. Additionally, we used the Wilcoxon rank sum test to determine the significance of the differences among the fitted model parameters: those fitted to diseased thoracic versus abdominal tissues, and those fitted to healthy (from Weisbecker et al., 2012) versus diseased tissues. More specifically on the latter, we compared the model parameters fitted to healthy descending thoracic tissue samples to those from diseased ascending thoracic samples because of the samples available. Here we adjusted the significance level with the Bonferroni-Holm correction. For all tests we assumed $p < 0.05$ to be significant.

2.2.5. Histology
In close proximity to the areas of tissue cut out for mechanical testing, we also cut out additional samples and stored them in 4% buffered formaldehyde solution (pH 7.4) for histological investigations at the Medical University Graz, Austria. We performed histological studies on formalin fixed and paraffin embedded cuts in the circumferential planes, normal to the axial directions of the samples. Following standard procedures we stained the samples with Hematoxylin and Eosin (H&E) to highlight cell cores, Picrosirius Red (PsR) to highlight fibrillar collagen and Elastica von Gieson (EvG) to highlight elastic fibers. We acquired images from a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan), set to either 20× or 100× magnification, using a Nikon Digital Sight DS-Fi1 digital camera.

3. Results

3.1. Material and damage parameters
We provide representative stress–stretch experimental data, model fits and corresponding evolutions of the damage variables:

![Fig. 1 - Representative stress–stretch experimental data, model fits and corresponding evolutions of the damage variables: (a, b) correspond to the thoracic sample T.2.1; (c, d) correspond to the abdominal sample A.8.2. Figs. 4 and 5 provide corresponding histological images.](image)
variables in Figs. 1–3. Fig. 1(a) and (b) corresponds to the thoracic sample T.2.1, and Fig. 1(c) and (d) corresponds to the abdominal sample A.8.2. Both samples show (strongly) anisotropic mechanical behavior and significant hysteresis, representative for all samples tested.

Figs. 2 and 3 show the range of inter- and intra-tissue responses for both the abdominal and thoracic samples, respectively. In particular, these figures show (Fig. 2 for all abdominal samples and Fig. 3 for all of thoracic samples) the model fits to the primary loading curves and the corresponding evolutions of the damage variables, where the top rows correspond to the axial directions and the bottom rows to the circumferential directions.

We provide the median values, interquartile ranges (Q₁ and Q₃) and coefficients of determination $R^2$ for material and damage parameters $\mu, k₁, k₂, \phi, \kappa, r_f$ and $m_f$ fitted to the intact, human thoracic and abdominal aortic aneurysmal tissues in Table 2. The measured mean thickness is 2.12 mm ± 0.43 for the abdominal samples and 1.98 mm ± 0.30 for the thoracic samples.

### 3.2. Statistical analysis

The statistical analysis demonstrates no correlation among the fitted model parameters generated from intact AAA tissues and the patient’s age or preoperative aneurysm diameter. Among all combinations tested, only BMI correlates in a statistically relevant manner with model parameters generated from AAA tissues, where both $k₁$ and $\kappa$ increase with increasing BMI. For the parameter $k₁$ the rank correlation coefficient $r_s$ equals 0.71 with a corresponding confidence interval CI of [0.09, 0.93], while for the parameter $\kappa$, $r_s$ and CI equals 0.78 and [0.23, 0.95], respectively. We find statistically relevant differences (at $p < 0.05$) between diseased abdominal and thoracic samples for $k₁$ and $\kappa$, where both are higher in abdominal samples.

### 3.3. Comparison with healthy tissues

Table 3 reviews the material parameters for healthy intact (three-layer composite) tissues, see Weisbecker et al. (2012).
When comparing intact aneurysmal tissues to healthy, intact (three-layer composite) tissues (Weisbecker et al., 2012) we find, for the human abdominal samples, statistically significant differences (at \( p < 0.05 \)) for \( \phi \) and the damage parameter \( m_f \). We obtain a fiber angle close to 45° for AAA tissues. The damage variable, an indicator for strain dependency, is smaller for AAA tissues. For the human thoracic aneurysmal tissues, the model parameters \( \mu \) and \( k_2 \) differ significantly from the corresponding parameters for healthy thoracic tissues. Here the shear modulus is higher, indicating a higher initial stiffness, while \( k_2 \) is smaller, indicating reduced exponential stiffening.

### 3.4. Histology

We provide representative histological images for the thoracic aneurysmal sample T.2.1 in Fig. 4 and for the abdominal aneurysmal sample A.8.2 in Fig. 5. The top row (a) and (b) of each figure shows the H&E staining, with cell cores stained dark purple; the middle row (c) and (d) shows the EvG staining, with elastic fibers highlighted black; and the bottom row (e) and (f) shows the PsR staining, with collagenous fibers stained red. In Fig. 5(d) we indicate significant calcifications with arrows. We generated images in the left columns with the magnification of the microscope set to 20 \( \times \), while on the right side this is set to 100 \( \times \). Figures in the same columns always show the same section of the samples.

### 4. Discussion

#### 4.1. Material and damage parameters

In the present work we tested intact, human thoracic and abdominal aortic aneurysmal tissues in uniaxial extension, although biaxial extension testing would better mimic the in vivo loading condition. We did this because uniaxial extension testing is better suited to higher (supra-physiological) loading and it allowed us to effectively test the relatively small samples that are available. We overcame the disadvantages of uniaxial testing for our application with...
Table 2 – Median values, interquartile ranges (Q₁, Q₃) and coefficients of determination R² for material and damage parameters fitted to the intact, human thoracic and abdominal aortic aneurysmal tissues.

<table>
<thead>
<tr>
<th>Patient #</th>
<th>μ (MPa)</th>
<th>k₁ (MPa)</th>
<th>k₂ (MPa)</th>
<th>ψ (deg)</th>
<th>κ</th>
<th>r₁</th>
<th>m₁</th>
<th>R²</th>
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<tbody>
<tr>
<td>T.1.1</td>
<td>0.046</td>
<td>7.92</td>
<td>0.000</td>
<td>50.7</td>
<td>0.145</td>
<td>1.00</td>
<td>0.026</td>
<td>0.94</td>
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<td>T.1.2</td>
<td>0.023</td>
<td>5.38</td>
<td>0.000</td>
<td>48.6</td>
<td>0.134</td>
<td>1.00</td>
<td>0.033</td>
<td>0.97</td>
</tr>
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<td>T.2.1</td>
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<td>1.92</td>
<td>0.000</td>
<td>46.6</td>
<td>0.135</td>
<td>1.93</td>
<td>0.002</td>
<td>0.97</td>
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<td>9.07</td>
<td>0.000</td>
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<td>0.256</td>
<td>1.00</td>
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</tr>
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<td>0.000</td>
<td>2.03</td>
<td>0.002</td>
<td>0.97</td>
</tr>
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<td>44.8</td>
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<td>0.005</td>
<td>0.97</td>
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<td>3.07</td>
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<td>0.98</td>
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<td>0.000</td>
<td>2.11</td>
<td>0.004</td>
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<td>2.500</td>
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<td>[Q₁, Q₃]</td>
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<td>[0.20, 4.52]</td>
<td>[0.000, 4.939]</td>
<td>[41.5, 46.5]</td>
<td>[0.005, 0.135]</td>
<td>[1.23, 2.94]</td>
<td>[0.003, 0.007]</td>
<td>[0.97, 0.98]</td>
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Table 3 – Median values, interquartile ranges (Q₁, Q₃) and coefficients of determination R² for material and damage parameters fitted to the healthy, intact (three-layer composite) human thoracic and abdominal aortic tissues, from Weisbecker et al. (2012).

<table>
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<th>Aorta</th>
<th>μ (MPa)</th>
<th>k₁ (MPa)</th>
<th>k₂ (MPa)</th>
<th>ψ (deg)</th>
<th>κ</th>
<th>r₁</th>
<th>m₁</th>
<th>R²</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>51.0</td>
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<td>1.59</td>
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<td>[0.24, 0.94]</td>
<td>[5.790, 34.79]</td>
<td>[46.8, 53.8]</td>
<td>[0.08, 0.28]</td>
<td>[1.25, 1.83]</td>
<td>[0.004, 0.011]</td>
<td>[0.95, 0.98]</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.019</td>
<td>5.15</td>
<td>8.64</td>
<td>38.8</td>
<td>0.24</td>
<td>1.10</td>
<td>0.013</td>
<td>0.98</td>
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<tr>
<td>[Q₁, Q₃]</td>
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<td>[2.58, 10.5]</td>
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<td>[35.8, 41.1]</td>
<td>[0.21, 0.25]</td>
<td>[1.00, 1.54]</td>
<td>[0.010, 0.019]</td>
<td>[0.93, 0.99]</td>
</tr>
</tbody>
</table>

a careful experimental protocol and model-fitting procedure (Holzapfel, 2006).

We used a pseudo-elastic constitutive model, introduced by Ogden and Roxburgh (1999), to fit the resulting mechanical data and study damage accumulating in the aortic aneurysmal tissues, causing softening during supra-physiological loading. A big advantage of this model is that we can split the fitting procedure into two parts. First we fitted the primary loading curve described by the elastic strain-energy function. Second, we used the resulting material parameters for the elastic response and fitted the unloading and reloading curves to determine the damage parameters.

Fig. 1(a) and (c) provides representative data gathered from mechanical testing as well as the fitted stress–stretch response. The pseudo-elastic constitutive model fits the data from both thoracic and abdominal tissues well. In both cases the material response is clearly anisotropic but the orientation of higher stiffness switches. In these particular samples the thoracic tissue (sample T.2.1) is stiffer in the axial direction while the abdominal tissue (sample A.8.2) is stiffer in the circumferential direction.

Fig. 1(b) and (d) provides the corresponding evolution of damage-induced softening, represented by the minimum of the damage variable. The model fits both the discontinuous softening caused by pre-conditioning in the physiological loading range and the softening damage caused during supra-physiological loading. Little is currently known about damage mechanisms within aneurysmal tissues, and whether or not softening that is still in the physiological range, sometimes referred to as preconditioning, should be distinguished from irreversible (inelastic) mechanisms occurring in the tissue structure. Hence, in this study we did not
set a threshold value to determine damage initiation. Balzani et al. (2006) calculated the strain-energy accumulated at an internal arterial pressure at 180 mmHg and defined this as the initial damage state, prohibiting the evolution of softening below this threshold. We were not able to define an objective threshold to distinguish between physiological and non-physiological damage and suggest that more specialized experiments and structural analysis should be performed. This remains a challenging research area because the damage mechanisms which ultimately lead to failure are not well understood.

Table 2 provides median values, interquartile ranges and coefficients of determination for material and damage parameters fitted to the intact, human thoracic and abdominal

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Fig. 4 – Representative histological images from the thoracic aneurysmal sample T.2.1: (a, b) H&E staining, (c, d) EvG staining and (e, f) PsR staining, from the entire sample in the left column (scale bar is 1000 μm) and from a magnified detail in the right column (scale bar is 100 μm). Fig. 1(a), (b) shows the corresponding mechanical test data, model fit and damage evolution.
aortic aneurysmal tissues. Note that nearly all patients had hypertension and hypercholesterolemia (Table A1), both known risk factors for cardiovascular diseases. Since the fitted model parameters are not normally distributed, we calculated the median values and the corresponding interquartile range to represent them properly, with the additional benefit that these values are less sensitive to outliers than the mean values and standard deviations.

The median fiber angle $\phi$ for the thoracic aneurysmal tissues is 44.8°, indicating a nearly isotropic behavior (N.B. at 45° the

Fig. 5 – Representative histological images from the abdominal aneurysmal sample A.8.2: (a, b) H&E staining, (c, d) EvG staining (significant calcifications indicated with arrows) and (e, f) PsR staining, from the entire sample in the left column (scale bar is 1000 μm) and from a magnified detail in the right column (scale bar is 100 μm). Fig. 1(c, d) shows the corresponding mechanical test data, model fit and damage evolution.
circumferential and axial directions appear equally stiff. However, the corresponding interquartile range from 41.5° to 46.5° shows high variability for the mean fiber orientation, from a circumferentially to an axially fiber-reinforced tissue. Sokolis et al. (2012) tested layer-separated human ascending TAAs and determined that medial and adventitial but not intimal layers were stiffer in the circumferential direction. Similarly, Pham et al. (2013) tested intact ascending aortic aneurysms under biaxial loading and determined that the tissue was stiffer in the circumferential direction. Conversely, Azadani et al. (2013) tested human ascending TAAs and proposed that there is no directional dependency, in accordance to the median value we found.

In contrast, we calculated a median value of $\varphi = 41.9^\circ$, with an interquartile range from 41.4° to 43.4°, for the abdominal aneurysmal tissues. Thus, abdominal aneurysmal tissues show a higher stiffness circumferentially, indicating a primary fiber orientation in this direction, also with less variation. Both Tong et al. (2011) and Vande Geest et al. (2006) reported similar findings. In the future, one could directly measure the fiber directions and the dispersions using methods proposed by, e.g., Schrießl et al. (2012a, 2013), and measurements which could be used to either validate the fitted median fiber angles or improve the accuracy of the fitted model by using the measured angles of fiber reinforcement as input data (Rodríguez et al., 2009).

Also related to the mechanical response of the collagen fibers, the fitted $k_2$ values for AAA tissues vary widely from zero (the strain-energy function from (5) is reduced to $\varphi_{ij}^2 = k_2(i \cdot j - 1)^2/2$, cf. Weisbecker et al., 2012) to relatively high values.

We also found large variations in the thickness within individual samples: the mean value was 2.12 mm $\pm$ 0.42 (mean $\pm$ SD) for the abdominal samples and 1.98 mm $\pm$ 0.3 for the thoracic samples. We also measured substantial variations in the thicknesses within individual samples, e.g., the mean thickness $\pm$ SD of four measured points from abdominal sample A.4 was 2.23 mm $\pm$ 0.46. Raghavan et al. (2006) reported similar variations in intra-sample thicknesses.

### 4.2. Statistical analysis

We used statistical methods to determine if our model parameters, fitted to data generated from testing of abdominal aortic aneurysmal tissues, correlated significantly with a patient’s age, BMI and preoperative aneurysm diameter. Among all combinations tested, only BMI correlated significantly with the model parameters generated from AAA tissues. No correlations were evident among the patient’s age and the model parameters, indicating that the effective age of the aneurysm, as introduced by Wilson et al. (2012), are more important in determining its mechanical properties. Additionally, pre-surgical aneurysm diameter has no significant influence on the model parameters. Our result is in accordance with the literature, see, e.g., Vorp (2007), and strengthens the theory that aneurysm diameter is an insufficient measure to recommend surgery. However, the relatively small sample population used in our study could have also caused us to miss significant correlations.

Regarding the significance of differences among the model parameters determined for thoracic versus abdominal aortic aneurysmal tissues, we found such a difference for the parameter $k_1$, which is larger for the abdominal tissues, indicating that the fibers contribute more to the mechanical response. The parameter $k_2$ also differs significantly and is larger for the abdominal tissues, indicating greater larger dispersion.

### 4.3. Comparison with healthy tissues

We found a significant difference in the parameters $\mu$ and $k_2$ between healthy and diseased thoracic tissue samples. Here $\mu$ is larger for aneurysmal tissues, indicating a higher stiffness in the low-strain region which could result from replacement of the elastic ground substance in favor of a collagenous network, with increased cross-linking of the collagen fibers (Lindeman et al., 2010). Lindeman et al. (2010) also showed that the collagen fibers of diseased tissues have reduced waviness and thereby appear stiffer at lower stretches. This is consistent with our findings that the parameter $k_2$ is lower which means a reduced exponential stiffening of the material occurs relative to healthy tissues. This could be due to a faster fiber recruitment at lower strains in the remodeled collagen network. We also found that the angles of collagen fiber reinforcement $\varphi$ vary significantly between healthy and diseased tissues. In both cases the aneurysmal tissues become less anisotropic, i.e., closer to $\varphi = 45^\circ$, which is in contrast to Vande Geest et al. (2006) who found anisotropy increased with aneurysm development. Median values for the damage parameter $f_f$ are higher for diseased abdominal tissues than for healthy tissues. This result indicates that less softening can occur in the diseased tissues. Here again we suggest that more research is necessary to identify the damage mechanism beyond normal physiological loading, because damage is the most likely trigger for remodeling processes, e.g., for restenosis (Timmins et al., 2011), which could also be related to stabilized aneurysms (Vorp, 2007). If significant calcification is evident in the tissue, most likely the softening can be interpreted as non-physiological damage (Mulvihill et al., 2013).

We used a statistical approach to determine the significance of differences among the model parameters fitted to healthy descending thoracic tissue samples (from Weisbecker et al., 2012) to those from diseased ascending thoracic samples. We know that there are differences in the mechanical properties between healthy ascending and descending thoracic aortic tissues (Rocchi et al., 2014), but because of the samples available, we assume that we can still capture trends in the changes of model parameters due to aneurysm growth.

Looking at the abdominal samples specifically, the diseased tissues have a median material parameter $k_2$ which is nearly zero such that the material is related to a quadratic strain-energy function rather than an exponential one. Additionally, the damage parameter $m_1$ is significantly lower for abdominal aneurysmal tissues than for healthy tissues, indicating that softening is induced at smaller strains.

Overall our results indicate that aneurysm development has a larger effect on the mechanical response of thoracic aortas than on abdominal aortas. Some remodeling processes, e.g., decrease in elastin, increase in collagen and atherosclerosis, happen in the healthy aorta as part of the normal aging process (Tsainis et al., 2013). Perhaps such
effects are more pronounced in the abdominal aorta, effects which would change the initial condition of the tissue for disease initiation (Wilson et al., 2012), i.e. a more remodeled initial tissue would appear to change less in disease.

4.4. Histology

The main goal of our histological investigations was to better understand the morphology of the aneurysms, and thus to better interpret our model parameters (and changes in these). Results indicate a significant difference between the TAA and the AAA tissues.

The TAA samples appeared overall more intact. Figs. 4 and 5 show similar signs of calcification and collagen remodeling, but these appear more severe in the AAA samples. Additionally, the content of both elastin (shown in Fig. 4(d) as black fibers) and smooth muscle cells (shown as cell cores in Fig. 4(b)) was higher in the thoracic samples.

In contrast, the AAA samples were highly remodeled, where the number of both elastic fibers and smooth muscle cells was drastically reduced and largely replaced by collagen fiber bundles, as shown in Fig. 5. In Fig. 5(b) the absence of smooth muscle cells is visible. In Fig. 5(c) the absence of elastin is visible over large areas of the sample, elastin which is replaced by collagen, as shown in Fig. 5(e). Signs of atherosclerosis presented as high calcification in all the AAA samples, shown as crystalline structures in the histological cuts, e.g., on the left side in Fig. 5(d), as indicated by arrows. In some cases, dramatic wall defects were also present.

Differences in the model parameters related to these structural differences. The collagen network which replaces softer elastin matrix corresponds to an increased mechanical influence of collagen, represented by a relatively higher value of the parameter $k_1$.

In all AAA samples, highly concentrated cell clusters, shown as accumulations of black dots on the right side of the tissue sample in Fig. 5(a), indicate the presence of inflammation. Such concentrated cell clusters were less common in the TAA samples and if present, not as distinct. Inflammation could play a key role in the development and growth of aneurysms, especially for AAA, according to Thompson (2005) and Shimizu et al. (2006).

4.5. Applications and shortcomings

Due to large patient variability, ‘one criterion for all’ approaches to estimate the rupture risk of aneurysms are unlikely to be accurate when applied to individuals. The main goal of this study was to obtain material parameters for abdominal and thoracic aortic aneurysmal tissues to facilitate FE analysis. Future methods for risk estimation of aneurysm rupture should tend toward patient’s specificity, e.g., FE simulations calibrated using medical images for building geometry, and constitutive models and fitted parameters such as those provided here. In light of the degeneration of active cells, the mechanical properties of highly remodeled aortic aneurysmal tissues might be fully characterized with passive testing alone.

Regarding our experiments, to our best knowledge no information about the influence of storage at $-20^\circ\text{C}$ on the mechanical properties of human aneurysm tissues is available. Adham et al. (1996) performed mechanical experiments with human tissue stored at $-135^\circ\text{C}$ and concluded that cryopreservation did not alter the mechanical characteristics of human descending thoracic aorta. Venkatasubramanian et al. (2006) investigated the changing mechanical behavior of femoral arteries from pigs due to freezing at $-20^\circ\text{C}$ and $-80^\circ\text{C}$ and concluded that major changes are only evident in the low-strain region of the mechanical response. In contrast, Chow and Zhang (2011) performed biaxial mechanical experiments on bovine thoracic aortas that had been stored at common storage temperatures ($4^\circ\text{C}$, $-20^\circ\text{C}$, or $-80^\circ\text{C}$) for three different time points (48 hours, 1 week, or 3 weeks). For a storage at $-20^\circ\text{C}$ the authors found no significant change in the low-strain regime but a significant shift in the high-strain regime of the mechanical response. Chow and Zhang (2011) concluded that there are changes within the tissue most likely due to intracellular ice formation. Additionally, O’Leary et al. (2014b) tested the same porcine aortic tissue samples before and after freezing/storage at $-20^\circ\text{C}$ and found no significant difference in the mechanical properties determined by experiments. Due to large variability in the fitted model parameters, naive use of the median parameters could lead to serious errors in FE simulations aimed at estimating stresses or aneurysm rupture risk. With improving imaging techniques, such as, e.g., optical coherence tomography (van Soest et al., 2010), optical coherence elastography (Kennedy et al., 2011), intravascular ultrasound (Suzuki et al., 2008) or B-mode ultrasound (de Groot et al., 2008), in vivo estimation of the tissue state might allow an accurate implementation of inhomogeneous material properties and therefore the range of material parameters might be of greater interest than median values. Kaladji et al. (2013) implemented a simplified attempt in this direction by varying Young’s modulus of arteries, in an FE simulation of endovascular insertion for aortic aneurysm repair, depending on an image-based estimate of the calcification level along the path of the guide wire. The determination and correct use of appropriate material parameters for simulations in soft tissue biomechanics, e.g., those of AAAs and TAAs, remains a major challenge in biomechanics (Miller and Lu, 2013).

In this study we neglected the active mechanical response of smooth muscle cells within the aortic tissues. In the highly remodeled AAA tissues this simplification should be acceptable. In the TAA tissues, where the muscle cells appeared largely intact, this simplification may be problematic. We would require more sophisticated constitutive models to account for the active response of the muscle cells, where biochemical processes are additionally considered, see, e.g., Murtada et al. (2012) and Murtada and Holzapfel (2014).

Finally, simulations based on the data provided here can only account for a single point in time, no long-term behavior and biological response can be predicted with our fitted constitutive model. To simulate the time evolution of an aneurysm, growth models (see, e.g., Satha et al., 2014) or remodeling models for collagen networks (see, e.g., Hadi et al., 2012) must be implemented. Nevertheless, for predicting failure and quantifying damage immediately after supra-physiological loading, e.g., stent insertion, the model provided here suffices.
Acknowledgments

We gratefully acknowledge the financial support of the European Commission under the 7th Framework Programme in the scope of the project SCATh – Smart Catheterization, Grant agreement number 248782.

Appendix A. Additional patient-specific data

<table>
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<th>Diabetic</th>
<th>Hypercholesterolemia</th>
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<th>Sport (hours/week)</th>
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‘NA’ = data not available.

REFERENCES


