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# Effects of Age on the Elastic Properties of the Intraluminal Thrombus and the Thrombus-covered Wall in Abdominal Aortic Aneurysms: Biaxial Extension Behaviour and Material Modelling

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**Abstract** *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.
*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.
*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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*Conclusion:* Our results suggest that thrombus age might be a potential predictor for the strength of the wall underneath the ILT and AAA rupture.


## 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%.[1] Several factors, including maximum AAA diameter, expansion rate of AAA,[2,3] peak wall stress,[3,4] and geometrical factors of the aneurysm,[5] 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.[6]

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.[7] Many previous studies suggest that the presence of the ILT alters the wall stress distribution and reduces the peak wall stress in AAA.[4,8–10] Thus, ILT serves as a mechanical cushion[4,9,11] and is usually found at the rupture site of the AAAs.[12] Vorp et al.[13,14] 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.,[15] 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[16] 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.[17,18] 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[19]; 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 $\pm$ 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[20] 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 $\times$ 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.[21]

ILT heterogeneity has been reported and highlighted by several representative studies.[15,16] 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 $\pm$ 1.0 °C by a heater-circulation unit (Ecoline E 200; LAUDA, LAUDA-Konigshofen, 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.[22] The non-zero stresses have the form

$$P_{\theta\theta}=\frac{f_\theta}{TX_L}, \quad P_{LL}=\frac{f_L}{TX_\theta}, \tag{1}$$