CHAPTER 3

Influence of aneurysm wall stiffness and the presence of intraluminal thrombus on the wall movement of an aneurysm
An in-vitro study

Submitted

ABSTRACT

**Objectives:** Purpose of this in-vitro study was to investigate the influence of aneurysm wall stiffness and of the presence of intraluminal thrombus (ILT) on aneurysm wall movement.

**Material & Methods:** Three latex aneurysms were used with different wall stiffness. The aneurysms, equipped with 20 tantalum markers, were attached to an in-vitro circulation model. Fluoroscopic roentgenographic stereo photogrammetric analysis (FRSA) was used to measure marker movement during 6 cardiac cycles. The radius of 3 circles drawn through the markers was measured. This was measured at three different systemic pressures: 100/70 mmHg, 120/90 mmHg and 160/90 mmHg.

To investigate the influence of ILT on wall-movement, we repeated the same experiment with one of the aneurysms. The aneurysm sac was then filled with one of two in E-moduli differing thrombus analogues (Novalyse 8 & 20) or with perfusate as a control.

**Results:** The amplitude of the wall movement (mm) increased significantly \( p<0.05 \) as the compliance of the wall increased (AAA1: \( 1.14 \pm 0.61 \) mm; AAA2: \( 1.03 \pm 0.47 \) mm; AAA3: \( 0.62 \pm 0.29 \) mm). The mean amplitude of the wall movement decreased \( p<0.05 \) as the stiffness (e-modulus) of the ILT increased (ILT_C: \( 0.48 \pm 0.28 \) mm; ILT_8: \( 0.38 \pm 0.22 \) mm; ILT_20: \( 0.30 \pm 0.12 \) mm).

**Conclusions:** Intraluminal thrombus has a “cushioning effect”. Wall movement (and theoretically wall stress) diminishes when the stiffness of the intraluminal thrombus increases. Compliance of the aneurysm wall influences wall movement. When the stiffness of the wall increases, the wall movement diminishes.
INTRODUCTION

Rupture of an abdominal aortic aneurysm (AAA) remains an important cause of death in people over 60 years of age. In this age group, the prevalence of an AAA ranges from 1.9–18.5%.1 In men, aged 65–74, there is a 10-year incidence of fatal and non-fatal aneurysm-ruptures of 0.84%.2 Many studies have been performed to investigate which factors determine whether an aneurysm ruptures. From a mechanical point of view, rupture of an AAA occurs when the local wall stress exceeds the local wall strength.3–6 However, many finite element studies have been done to investigate the influence of different determinants of wall stress. The amount of stress depends on several factors such as: local AAA diameter, AAA-geometry, presence of intraluminal thrombus (ILT), ILT-thickness and wall characteristics.5 Several studies have shown that the presence of ILT can diminish the wall stress of an aneurysm by 24–51%.6–9 It is thought that ILT might have a sort of mechanical “cushioning effect”. Mower et al. state that the protective effect is dependent on its elastic modulus: stiffer thrombi stretch less and “absorb” more wall stress than compliant thrombi and are thus more efficient in reducing wall stress.9 This was underlined in a recent study of Speelman et al.10

Purpose of this in-vitro study was to investigate if the presence and elastic properties of intraluminal thrombus (ILT) influence the extent of wall movement. Our hypothesis is that ILT absorbs the energy of the circulation and thereby has a mechanical protective effect on wall movement. Furthermore, we investigated whether wall stiffness has an influence on wall movement. This study focuses on the mechanical effects and will not look into the biochemical effect of thrombus or wall-stiffness.

MATERIALS AND METHODS

In-vitro circulatory system
An in-vitro circulation model (Fig. 3.1), which was validated and described previously, was used.11–13 A plexiglass box containing a latex aneurysm was connected to the in-vitro circulation model. A starch solution with the same viscosity as blood was used in this set-up.11

The systemic pressure was measured by using a Datascope 2000 digital pressure monitor [Datascope Corporation, Paramus, N. J., U.S.A].

Latex aneurysm models
In this experiment fusiform latex aneurysm models with different wall stiffness were used. Aneurysms of 4 layers (AAA1), 8 layers (AAA2) and 12 layers (AAA3) latex were produced. In the physiological pressure range [60–160 mmHg] the compliance for the
4-, 8- and 12-layer latex aneurysms were respectively 0.23–0.89 ml/mmHg, 0.05–0.16 ml/mmHg and 0.04–0.07 ml/mmHg. These compliances are comparable to the in vivo situation. The wall-thickness’s were respectively 1.2mm, 1.9mm and 2.5mm.

Twenty tantalum markers (0.8mm) were placed between the latex layers for fluoroscopic roentgenographic stereo photogrammetric analysis (FRSA) -measurements. The markers were placed in 5 circles of 4 markers on the proximal (A; 1–4) and distal neck (E; 17–20), on the widest part (C; 9–12) and between the necks and the maximum diameter (B; 5–8 and D; 13–16) of the AAA (Fig. 3.2). The maximum inner radius of the aneurysm was 18.0 mm and the inner radius of the proximal and distal aorta was 8.0 mm.

**Presence of intra-luminal thrombus.**

To investigate the role of intraluminal thrombus (ILT) on wall-movement, a thrombus analogue was inserted in the 12 layer latex aneurysm. These thrombus analogues [Novalyse, Kobato Nova Products BV, Ootmarsum, The Netherlands] were a polymer product and had the same mechanical properties (E-modulus) as human thrombus. Two different types of analogue were used: Novalyse ST8 and ST20. The E-modulus of these analogues were 27 and 60 kPa. Human thrombus has a mean E-modulus of 37 (Fig. 3.1 A schematic representation of the circulation set-up and the FRSA set-up. The circulation set-up consisted of a pressure measuring device (A), an artificial heart driver (B), left ventricle (C), an open reservoir (D), a ball valve (E), an air chamber (F), an air tight pressure box with an latex aneurysm (G), a systemic pressure sensor (H) and a blood pressure cuff (I). The FRSA set-up consisted of the roentgen foci (R1 and R2), the detectors (D1 and D2). Their relative positions are known by calibration of the setup. Markers give projections P1 and P2 on the detectors. With a calibrated setup, projection lines can be reconstructed. Calculation of intersection M of these projection lines in space gives the position of a marker.)
Effect of ILT and Compliance on Wall-Stress

±15) kPa. Novalyse consists of two liquid components, namely STA and STB. Once the components are put together in the proportion of weight 2 STA:1 STB, Novalyse hardens in a few minutes at 20°C. As the analogue was very crumbly and could easily flow away, the analogue was kept in place by a thin walled, highly compliant, latex tube. A control measurement was done with perfusate between the excluding tube and aneurysm wall to make sure that this thin walled tube did not influence the measurements. Earlier research has shown that pressure is transmitted through such a compliant tube. The following references were used for the models: ILT_8 for the Novalyse ST8, ILT_20, for the Novalyse ST20 and ILT_C for the control measurement.

FRSA Set-up
Wall motion [mm] of the aneurysm wall was measured by using fluoroscopic roentgenographic stereo photogrammetric analysis (FRSA). FRSA is used to calculate the point of intersection of two projection lines of a marker in space by using calibrated stereo roentgenographic imaging (Fig. 3.1). This way the 3D position of a marker can be traced in time with very accurate precision. The frame rate was set at 30 images / second. The image pairs were analyzed by using model-based RSA software [ModelBased-RSA 3.21; MEDIS Specials, Leiden, The Netherlands] to calculate the relative three-dimensional marker positions. FRSA has an accuracy of 0.003 ±0.0019mm on marker motion detection. Circles were fitted through the markers (Fig. 3.2) using routines implemented

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Fig. 3.2 The latex aneurysm (length 37.5mm; widest Ø 15mm; neck Ø 37.5mm) was equipped with 20 tantalum markers (Ø 0.8mm). The markers were placed in a 5 circles (A-E) of 4 markers at respectively the proximal neck (A; 1–4), on a quarter (B; 5–8), the widest part (C; 9–12), on three-quarter (D; 13–16) and the distal neck (E; 17–20). The red arrows indicate the direction of the circulation.
in Matlab r2006B [The Mathworks, Natrick, USA]. The wall-motion of the aneurysms was calculated by the determination of the amplitude of the radius change of each circle. The amplitude was calculated by subtracting the minimal circle radius from the maximum measured circle radius during each pulsatile cycle.

**Measurements & Statistics**

The amplitude was measured with different pressure settings: 100/70, 120/90 and 160/90 mmHg. The circulation pump ran on a frequency of 70 b.p.m. For each pressure setting, the model ran 6 cardiac cycles.

![Graphs showing amplitude of radius changes](image)

**Fig. 3.3** Mean amplitudes of the radius of the rings during a heart cycle in the wall stiffness experiments. The bars depict the mean amplitude of the radius (mm), while the whiskers depict the standard deviation of the amplitude measurements. In the left above section the mean amplitude of all rings results of the wall experiments are shown, while the other graphs show the results of the proximal (A), middle (C) and distal ring (E). AAA1 @ 160/90 mmHg is missing due to a near rupture of the latex model at this pressure setting.
The results were analyzed with the statistics program SPSS 16.0 for Windows [SPSS Inc, Chicago, Ill, USA]. A 2-way Anova was done to compare the differences in amplitudes between the three different wall-stiffnesses (AAA1, AAA2, and AAA3). The parameters mean pressure and number of layers were investigated for its effects. This test was also used to compare the amplitudes of wall motion of the models with ILT (ILT_C, ILT_8, and ILT_20). With the ILT model, the mean pressure and the type of ILT were investigated.

RESULTS

Wall-Stiffness and wall movement

Both the factors “number of layers” and “mean pressure” had a significant effect on the amplitude of wall movement (Fig. 3.3 & Table 3.1). The amplitude of the radius of the fitted rings increased significantly (p<0.05) with AAA2 & AAA3, when the mean pressure in the circulation was increased. With the 4 layer latex aneurysm (AAA1), it was not possible to do a measurement at a pressure of 160/90 mmHg, as the aneurysm nearly

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<th>Wall measurements</th>
<th>Mean difference (1-2) [mm]</th>
<th>S.E. [mm]</th>
<th>Sig (p)</th>
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<td>1. AAA1 vs 2. AAA2</td>
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<td>1. AAA1 vs 2. AAA3</td>
<td>0.786</td>
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<td>1. 100/70 vs 2. 160/90</td>
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<td>1. 120/90 vs 2. 160/90</td>
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<td>1. 120/90 vs 2. 160/90</td>
<td>-0.408</td>
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Table 3.1. Overview table of 2-way Anova results. Per experiment first the overall effects of a variable are stated, after which the pairwise comparison between the variables is noted.
ruptured, with increasing systemic pressure. The mean amplitude of the wall movement decreased significantly (p<0.05) as the thickness of the aneurysm wall increased (AAA1: 1.14 ± 0.61mm; AAA2: 1.03 ± 0.47mm; AAA3: 0.62 ± 0.29mm) (Fig. 3.3).

**Intraluminal Thrombus (ILT) and wall movement**

The factors “Intraluminal thrombus” and “mean pressure” had a significant effect on the amplitude of wall movement (Fig. 3.4 & Table 3.1). When the mean pressure in the circulation was increased, the mean amplitude of the radius of the fitted rings increased significantly (p<0.05) with all aneurysms. The mean amplitude of the wall movement decreased (p<0.05) as the stiffness of the ILT increased (ILT_C: 0.48 ± 0.28mm; ILT_8: 0.38 ± 0.22mm; ILT_20: 0.30 ± 0.12mm) (Fig. 3.4).

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**Fig. 3.4** Mean amplitudes of the radius of the rings during a heart cycle in the intraluminal thrombus experiments. The bars depict the mean amplitude of the radius (mm), while the whiskers depict the standard deviation of the amplitude measurements. In the left above section the mean amplitude of all rings results of the wall experiments are shown, while the other graphs show the results of the proximal (A), middle (C) and distal ring (E).
DISCUSSION

This in-vitro study shows that the extent of aneurysm wall-movement depends on the presence of intraluminal thrombus (Fig. 3.4 & Table 3.1). The presence of intraluminal thrombus seems to have a “cushioning effect” in this in-vitro model. As the e-modulus of ILT increases, the mean amplitude of wall-motion decreases. When the amplitude of the wall movement decreases, the maximum wall radius at the peak of the systole will diminish. Considering the law of Laplace \( (\sigma = pr/2t) \), the peak wall stress \( (\sigma) \) will be lower when the maximum wall radius \( (r) \) is lower, considering the pressure \( (p) \) and the wall thickness \( (t) \) to be equal. The graph of ILT_8 and ILT_20 shows that the amplitude of the wall movement diminishes as the e-modulus increases (Fig. 3.4), thereby decreasing the maximum wall radius and thereby theoretically the peak wall stress.

The mean amplitude of the wall movement diminishes as the compliance of the wall decreases (Fig. 3.3 & Table 3.1). Because of the high compliance of the 4 layer latex aneurysm (AAA1), it was not possible to do a measurement at a pressure of 160/90 mmHg, as the aneurysm nearly ruptured, with increasing systemic pressure.

The present study is the first in-vitro study to analyze the role of wall stiffness and thrombus composition of vessel wall movement. The results correspond well with the results from earlier studies. With regard to the stress reduction by ILT, Inzoli et al. showed in a finite element analysis model that the presence of thrombus can lead to stress reduction of 30%.

Also using computer modelling, Mower et al. showed that stiff thrombus (E-modulus 1.0 MPa) can diminish the wall stress with even 51 %, while with more compliant thrombus (E-modulus 0.2 MPa) a stress reduction of 25% takes place. They stated that ILT has protective effect on wall stress, which is dependent on its elastic modulus: stiffer thrombi stretch less and “absorb” more wall stress than compliant thrombi and are thus more efficient at reducing wall stress. Mower en Inzoli, both used simple models with an axisymmetrical anatomy to investigate these effects. Wang et al. used patient specific 3D AAA geometries from patient CT-images. In their study, they show that in life like anatomies, the peak wall stress is reduced by the presence of ILT with 6 to 38%. Li et al, saw in a similar study with CT-data from 20 patients a significant stress reduction by ILT of 5–43% (median 24%). In a recent study, Speelman et al. showed in a computer model, using a simplified axisymetrical aneurysm model, that the amount of stress-reduction is dependent on the stiffness and the relative amount of intraluminal thrombus.

The present study underlines the fact that the presence of intraluminal thrombus diminishes the amplitude of the wall motion significantly \( (p<0.05) \) (Table 3.1). This diminishment in wall-motion can theoretically lead to a diminished amount of wall-stress. From a mechanical point of view, the chance of aneurysm rupture diminishes, when the stress on the aneurysmal wall diminishes.
This beneficial effect of thrombus on the biomechanical behavior of an aneurysm may lead to new treatment options for aortic abdominal aneurysms. Several groups are investigating the option of filling the aneurysm sac with different kinds of injectable polymers.\textsuperscript{13, 20–22} The presence of such a polymer will diminish the stress exerted on the aneurysmal wall, if the polymer is stiffer than the thrombus in the sac.

The analysis of our results showed that the mean pressure has a role in the amount of wall-movement as well. The mean pressure had a significant effect on the mean amplitude, in both the wall stiffness ($p<0.05$) and the intraluminal thrombus experiments ($p<0.05$). In the wall stiffness group, with aneurysm AAA1 the amplitude did not increase, but decreased with the pressure increase of 100/70 to 120/90 mmHg. This is due to the fact that with the pressure of 120/90 mmHg, the maximum diameter was reached, leading to a smaller amplitude of diameter, as no further stretch of the latex was possible. As stated above, when attempting to increase the pressure to 160/90 mmHg, the model nearly ruptured. Table 3.1 shows a significant decrease in amplitude by diminishing the systemic pressure from 160/90 mmHg to 120/90 mmHg or 100/70 mmHg ($p<0.05$), while there was no significant difference between the amplitudes at 120/90 and 100/70 mmHg. This result will probably not be due to the difference in the mean pressure, but more likely caused by the larger pulse pressure with the 160/90 mmHg. However, this result shows there is a basis for a hypertension prevention treatment policy with (R)AAA patients. As stated above, wall stresses will be lower as the maximum wall radius will be lower.

**Limitations of the study**

As every in-vitro experiment, this set-up is a simplification of the in-vivo situation. The anatomy of the used aneurysm is very straightforward as it is a perfect spherical object. This differs from the in-vivo situation where aneurysms are asymmetrical. The scope of this research was to investigate the influence of intraluminal thrombus and wall-thickness on wall motion and wall-stress. As earlier research has shown that geometry has a large influence on wall stress\textsuperscript{5}, we have chosen to use a simple spherical anatomy.

The thrombus analogue had a standardized stiffness and configuration, while in-vivo the stiffness of the thrombus and its configuration may differ enormously for each aneurysm, due to heterogeneity. In the present study standardized variables were used, as otherwise all the different variables might have influenced the outcome.

Other researchers used force gauges in similar experiments, to measure the wall stress with aneurysms with different compliances.\textsuperscript{23} We decided not to use force gauges as the presence of these gauges influences the local stiffness of the aneurysm and thereby could influence the results. Furthermore, these gauges are difficult to attach to latex aneurysm and are prone to malfunction.\textsuperscript{23} The FRSA technique is very accurate for motion measurements.
and has a mean measurement error of only 0.003mm.\textsuperscript{15} Using this technique we could accurately measure the location of each marker and calculate the radius of the aneurysm sac.

**Clinical Relevance**

Rupture of abdominal aortic aneurysms occurs when aneurysmal wall stresses exceed aneurysmal wall strength.\textsuperscript{5, 24} Finite element models are designed to investigate aneurysmal wall behavior and the influence of aneurysm characteristics such as intraluminal thrombus and wall thickness.\textsuperscript{5–9} This study is the first study to show the influence of these characteristics in a controlled in-vitro situation. The results underline the mechanical cushioning effect of ILT, which potentially can be used to improve future aneurysm treatment concepts, such as aneurysm sac filling.\textsuperscript{13, 22} Furthermore the results of this study can be used to optimize computer simulations of aneurysmal wall behavior.

**CONCLUSIONS**

This in-vitro study shows that the extent of aneurysm wall-movement (and theoretically wall-stress) depends on the stiffness of the aneurysm wall and the presence of intraluminal thrombus. The presence of intraluminal thrombus diminishes wall movement and is dependent on the composition of the thrombus. The wall motion diminishes as the e-modulus of the ILT increases. This concept may lead to better understanding of aneurysmal biomechanics and can be used to develop new AAA treatment concepts.

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REFERENCES


