

## EXPERIMENTAL CHARACTERIZATION OF THE MECHANICAL PROPERTIES OF THE ABDOMINAL AORTIC ANEURYSM WALL UNDER UNIAXIAL TENSION

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Although many researchers have made the assumption that the abdominal aortic aneurysm (AAA) wall behaves as an incompressible and isotropic material, the experimental evidence for it is insufficient. Hence, the assumptions about the incompressibility and isotropy of the AAA wall were verified through analysis of stretch ratios of samples excised from the aneurysms walls. The stretch ratios were calculated on the basis of a real-time analysis of geometric dimensions of samples subjected to uniaxial tension. It was proved that the walls of abdominal aortic aneurysms can be modelled as an incompressible and isotropic material. Using histological techniques, the assumption concerning the negligence of shear stress in the analysis of AAA wall stresses was indirectly validated. The results were incorporated into a hyperelastic constitutive equation.

*Key words:* abdominal aortic aneurysm, incompressibility, isotropy, shear stress

### 1. Introduction

The abdominal aortic aneurysm (AAA) is a permanent and progressing dilation of the abdominal aorta by at least 50% as compared with its normal diameter (Sakalihan *et al.*, 2005; Li and Kleinstreuer, 2006). It results from the pathological multifactor remodelling the aortic wall connective tissue caused by enzymatic degradation of the main load-bearing components, i.e. elastin and collagen fibres (Brady *et al.*, 2004; Longo *et al.*, 2005). The initiation and development of an AAA results in significant changes in the mechanical properties of the abdominal aorta wall (DiMartino *et al.*, 2006; Geest *et al.*, 2006a; Kobielarz *et al.*, 2008). This means that a proper theoretical basis is essential for description of the mechanical properties of AAA walls.

Despite the intensive development of models and constitutive equations for pathologically unaffected blood vessels modelled as poroelastic materials (Simon *et al.*, 1998; Johnson and Tarbell, 2001), viscoelastic materials (Veress *et al.*, 2000; Holzapfel *et al.*, 2002) or pseudoelastic materials (Fung, 1967; Chuong and Fung, 1986), for behaviour of AAA walls behaviour under mechanical loads models based on the linear theory of elasticity (Mower *et al.*, 1997; DiMartino *et al.*, 1998; Vorp *et al.*, 1998) or on the law of Laplace (Elger *et al.*, 1996; Hall *et al.*, 2000) are still commonly used. The application of the Laplace law to the assessment of the mechanical properties of AAA walls under mechanical loads is incorrect for two reasons. Firstly, the AAA's geometry does not correspond to a thin-walled cylinder or a sphere with a single curvature radius, for which the Laplace law holds true. Each aneurysm has a different shape, a complicated geometry with a different degree of eccentricity, and a variable wall thickness (Damme *et al.*, 2005; Vorp and Geest, 2005). Secondly, the AAA diameter is not the only determinant of wall stresses (Vorp *et al.*, 1998; Geest *et al.*, 2006b). Neither is the application of the linear theory of elasticity to the assessment and analysis of the mechanical properties of AAA walls proper since this material stress-strain characteristic has been shown to be nonlinear (Raghavan *et al.*, 1996; Kobielarz *et al.*, 2004). Therefore, the mechanical properties of aneurysms walls should be

assessed on the basis of the nonlinear theory of elasticity. Moreover, the AAA wall is a material which is subjected to large strains (amounting to 20%-40%) prior to its failure (He and Roach, 1994; Raghavan *et al.*, 1996). Hence, it is necessary to use the theory of large strains in order to model the behaviour of AAA walls under mechanical loads. The few constitutive equations derived from the nonlinear theory of elasticity, i.e. hyperelastic models (Yamada *et al.*, 1994; Raghavan and Vorp, 2000) are used assuming AAA wall incompressibility and isotropy and neglecting shear stresses without experimental evidence however. Therefore, the main objective of this research is to verify the *a priori* assumptions about abdominal aortic aneurysms walls for a large group of preparations and to evaluate the application of the results in a hyperelastic constitutive equation taking the theory of large strains into account.

## 2. Material and method

### 2.1. Test material

The test material had the form of 96 AAA wall specimens intraoperatively taken from the anterior parts of the vessel. The material was stored in a physiological salt solution at a temperature of 4°C until testing (no longer than 12 h). The samples were obtained by permission of Bioethical Commission at the Medical University of Wrocław, and the studies were conducted in accordance with the established procedures of preparation and storage of the biological material.

### 2.2. Assumptions

The assumption about the incompressibility and isotropy of AAA walls leads to many simplifications in the constitutive equation formulas and easier stress analysis. The material incompressibility and isotropy assumptions are correct when proper conditions are satisfied, i.e. the product of the stretch ratios is constant and equal to 1 ( $\lambda_1\lambda_2\lambda_3 = 1$ ) and the stretch ratios in directions perpendicular to the exciting force are equal to each other ( $\lambda_2 = \lambda_3$ ). The assumptions were verified for uniaxial tension (at a constant rate of 2 mm/min) of samples excised from AAA walls in two directions orthogonal to the vessel long axis, i.e. in the circumferential direction (AAA<sub>c</sub>) and the longitudinal direction (AAA<sub>l</sub>). The excised quasi-planar samples, having an initial width  $s_0$  of 5.0 mm, were mounted in a testing machine (*Synergie 100*, MTS, Fig. 1) by means of jaw chucks. The initial length  $l_0$  of each sample was 25 mm (at a deviation less than 0.5 mm). The Lagrangian stretch ratios in the three orthogonal directions ( $\lambda_1, \lambda_2, \lambda_3$ ) were calculated from the geometric dimensions of the samples recorded in real time in the course of the uniaxial tension test with a frequency of 5 Hz by a videoextensometer (*ME 46-350*, *Messphysik*).



Fig. 1. Measurement set-up: testing machine (*Synergie 100*, MTS) and videoextensometer (*ME 46-350*, *Messphysik*)

The uniaxial tensile test was preceded by pre-stretching: the sample was preloaded to 10% of its initial length and then unloaded to zero. The full cycle was repeated three times since the preliminary tests showed that the stress level stopped decreasing after three full stress cycles and the material behaved in a repeatable way during the further cyclic loading and unloading.

The concept of neglecting shear stress in the analysis of AAA wall stresses is based on the commonly held view today that degeneration of the inner layer, containing endothelial cells sensitive to shear stress, takes place in the walls of abdominal aortic aneurysms (Holmes *et al.*, 1995). The presence of degenerative changes in the inner layer of AAA walls has been proven through a histological analysis. Samples about 10 mm<sup>2</sup> of the full vascular wall thickness in size were excised from the test material, fixed in a 4% aqueous solution of formalin washed under running water for 24 hours and dehydrated through immersion in alcoholic baths with an ever higher concentration (from 70% alcohol to absolute alcohol). Then the samples were immersed in sodium benzoate for 24 hours. The material prepared in this way was embedded in paraffin. The paraffin block containing the material was cut into 5  $\mu$ m thick slices by means of a *Mikron HM315 (Zeiss)* microtome. The samples were dyed in two ways: with haematoxylin and eosin (H&E) and by Van Gieson's method. The histological preparations were viewed under light microscope *AxioImager M1m (Zeiss)*.

### 2.3. Statistical analysis

The results were presented as averages with standard deviations ( $X \pm SD$ ). The statistical analysis was performed using Student's t-test for dependent samples (Statistica 8.0, StatSoft). The tests were carried out assuming the limit significance level ( $p$ ) of 0.05.

## 3. Results

### 3.1. Verification of assumptions

The average stretch ratios in two directions perpendicular to the exciting force, and the product of the stretch ratios in three orthogonal directions were calculated (Table 1).

**Table 1.** Product of the stretch ratios  $\lambda_1\lambda_2\lambda_3$  and values of the coefficients:  $\lambda_2$  and  $\lambda_3$  for samples excised from AAA walls in the circumferential direction (AAA<sub>c</sub>) and longitudinal direction (AAA<sub>l</sub>) relative to the vessel long axis

AAA <sub>c</sub>			AAA <sub>l</sub>		
$\lambda_1\lambda_2\lambda_3$	$\lambda_2$	$\lambda_3$	$\lambda_1\lambda_2\lambda_3$	$\lambda_2$	$\lambda_3$
$0.98 \pm 0.21$	$0.95 \pm 0.04$	$0.94 \pm 0.04$	$0.99 \pm 0.25$	$1.00 \pm 0.06$	$0.98 \pm 0.04$

The obtained results show that the AAA wall incompressibility assumption (regardless of the sample excision direction) is correct since the product of stretch ratios is equal to approximately 1 in each considered case. The results also indicate that aneurysms walls under uniaxial tension behave as an isotropic material since the statistical analysis did not show any statistically significant differences in the results ( $\lambda_2$  vs.  $\lambda_3$ ) within the particular groups (for AAA<sub>c</sub>:  $p = 0.43$ ; for AAA<sub>l</sub>:  $p = 0.19$ ).

The histological analysis revealed disorders in the laminar structure of the AAA walls (Fig. 2). Most of the analysed walls (63%) were found to be devoid of the inner layer. In the cases when the inner layer is not completely atrophied, there are series degenerative changes whose principal feature is the lack of any visible layer of endothelium cells.

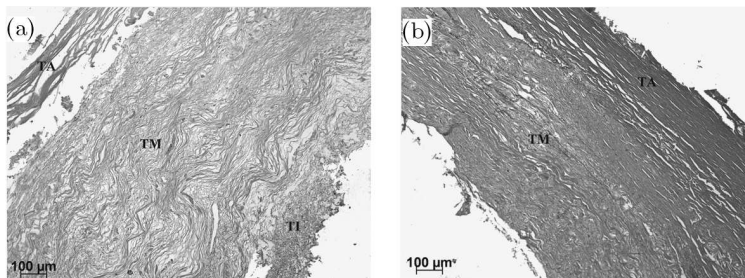


Fig. 2. Histological images of AAA walls: (a) with degenerated inner layer (TA – adventitia, TM – media, TI – intima), using H&E staining and (b) with atrophy of the inner layer, using Van Gieson’s staining

**3.2. Model and constitutive equation**

The behaviour of AAA walls under uniaxial loading was described using the generalized neo-Hookean model. For incompressible hyperelastic materials, the strain energy density function in the neo-Hookean model depends on the first invariant ( $I_1$ ) of the Cauchy-Green deformation tensor as follows

$$\Psi = c(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) = c(I_1 - 3) \tag{3.1}$$

where  $c$  is an equation parameter;  $I_1$  – first invariant of the right Cauchy-Green transformation tensor.

The constitutive equation for such a material assumes the form

$$\sigma = -pI + 2\frac{\partial\Psi}{\partial I_1}B \tag{3.2}$$

where  $\sigma$  is the Cauchy stress tensor;  $p$  – Lagrange multiplier;  $I$  – identity tensor;  $B$  – left Cauchy-Green deformation tensor.

When the incompressibility ( $\lambda_1\lambda_2\lambda_3 = 1$ ) and isotropy ( $\lambda_2 = \lambda_3$ ) of the considered material is introduced, from equation (3.2) one can derive the following relation describing the Cauchy stress tensor component in the exciting force direction ( $\sigma_1$ )

$$\frac{d\Psi}{dI_1} = \frac{\sigma_1}{2(\lambda_1^2 - \lambda_1^{-1})} \tag{3.3}$$

Raghavanand and Vorp (2000) found that the dependence between  $d\Psi/dI_1$  and  $I_1 - 3$  has a linear character. Ultimately, for uniaxial tension, the constitutive equation proposed by Raghavan and Vorp (2000) assumes the form

$$\sigma_1 = [2\alpha + 4\beta(\lambda_1^2 + 2\lambda_1^{-1} - 3)](\lambda_1^2 - \lambda_1^{-1}) \tag{3.4}$$

Taking into account the relation for the normal component of the Green strain ( $E_1$ ) tensor in the exciting force direction, on the assumption that the shear components of the deformation gradient tensor are insignificant (Van Bavel *et al.*, 2003; Geest *et al.*, 2006b), one gets

$$E_1 = \frac{1}{2}(\lambda_1^2 - 1) \tag{3.5}$$

where  $\lambda_1$  is the stretch ratio in the exciting force direction.

Hence, the following form of the constitutive equation is

$$\sigma_1 = \left\{ 2\alpha + 4\beta \left[ (2E_1 + 1) + 2\sqrt{2E_1 + 1} - 3 \right] \right\} \left[ (2E_1 + 1) - 2\sqrt{2E_1 + 1} \right] \tag{3.6}$$

The stress-stretch ratio curves obtained from the uniaxial tension test were described using constitutive equation (3.4), while the stress-strain curves were described using constitutive equation (3.6). Equations (3.4) and (3.6) fit the curves with a very good approximation:  $R_{min}^2 = 0.962 \pm 0.011$  and  $R_{min}^2 = 0.969 \pm 0.027$ , respectively. The degree of fitting was analyzed using the *Microcal Origin 7.0* software. The approximating function was determined for all the considered cases and the averaged data (Figs. 3 and 4).

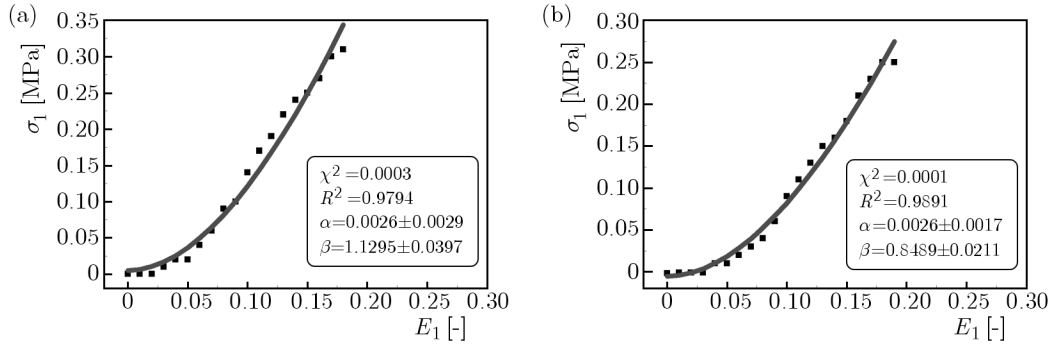


Fig. 3. Stress-stretch ratio curves described by constitutive equation (3.4) for samples excised from walls of tested blood vessels: (a)  $AAA_c$ ; (b)  $AAA_l$

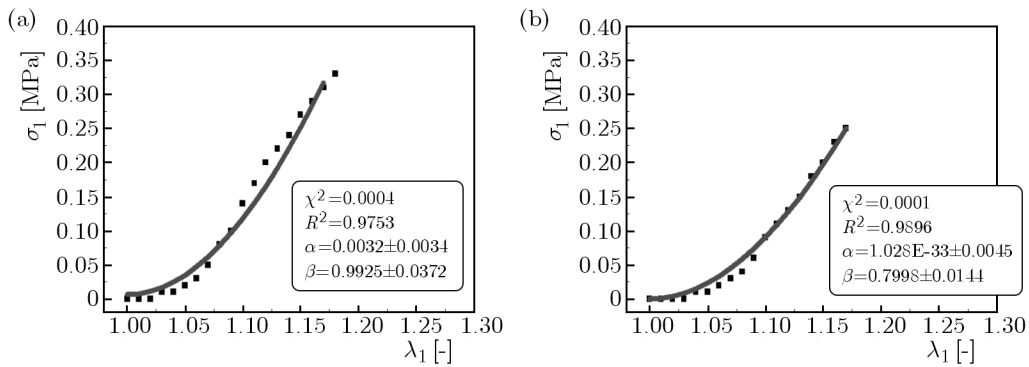


Fig. 4. Stress-strain curves described by new constitutive equation (3.6) for samples excised from walls of tested blood vessels: (a)  $AAA_c$ ; (b)  $AAA_l$

Coefficients  $\alpha$  and  $\beta$  are the best-fit material parameters of constitutive equations (3.4) and (3.6), and they did not significantly differ statistically between the sample excision directions. The values of the material constants obtained by the authors are lower in comparison with the ones reported by Raghavan and Vorp (2000), although for the coefficient  $\beta$ , the order of magnitude is the same. Whereas the constant  $\alpha$  obtained by the authors is at least two orders of magnitude lower for both models (3.4) and (3.6).

#### 4. Discussion

In recent years, significantly increased interest in experimental studies of mechanical properties of biological tissues, including hard (Kot *et al.*, 2011; Nikodem, 2012) and soft (Pezowicz, 2010; Źak *et al.*, 2011) tissues. Now, to describe the behaviour of soft tissues under different conditions of mechanical loading, a nonlinear theory of elasticity (Holzapfel, 2000; Humphrey, 2002) is commonly used. For description of pathologically altered tissue, usually adjusted models previously developed for tissue without pathological changes are incorporated. Assumptions of adapted constitutive models require verification, however. Hence, the assumptions of incompressibility,

isotropy and shear stress in the abdominal aortic aneurysm wall were analysed, because many researchers have made the assumption without the experimental evidence.

In the literature, it is commonly assumed that under mechanical loads, the walls of abdominal aortic aneurysms, similarly as those of healthy vessels, are almost incompressible (Thubrikar *et al.*, 2001; DiMartino *et al.*, 2006; Raghavan *et al.*, 2006). The assumption about the blood vessel wall incompressibility was introduced by Carew *et al.* (1968) who proved that under physiological strains, the walls of blood vessels behave as an incompressible material. The incompressibility assumption is based on the principle of conservation of a structure volume during the deformation of its material (Vito and Dixon, 2003). The incompressibility assumption makes sense in the case of biological tissues containing large amounts of water since water, is incompressible under physiological pressures (Vito and Dixon, 2003). This assumption is also valid for blood vessel walls which show negligible permeability to water (Chuong and Fung, 1986; Holzapfel and Ogden, 2003). Also in the present work, it was demonstrated that the walls of abdominal aortic aneurysms can be regarded as an incompressible material. The growth of an AAA does not result in a loss of the vascular wall ability to maintain its volume constant as the vessel structure is subjected to deformation (uniaxial loading). However, in the case of AAA walls showing signs of rupturing, permeability certainly increases, which may be the reason why the standard deviation was found to be quite high. The influence of the degree of advancement of the disease on the incompressibility of AAAs should be the subject of further research.

The walls of healthy blood vessels are treated as anisotropic materials because of their complex and heterogeneous structure (Holzapfel and Weizsacker, 1998; Geest *et al.*, 2004). It is known that the mechanical properties of a healthy blood vessel depend mainly on its middle layer (Humphrey, 1995; Ogden and Schulze-Bauer, 2000). Histologically, the middle layer is a highly organized three-dimensional heterogeneous network built of three main structural components (elastin fibres, collagen fibres and smooth muscle cells), but as Ogden and Schulze-Bauer (2000) research shows, under mechanical loads, the middle layer behaves as a homogenous material. Moreover, Stergiopoulos *et al.* (2001) showed that the middle layer in the pig aortic wall is characterized by a uniform distribution of matrix proteins and smooth muscle cells, and similar mechanical properties along its entire thickness. Hence, in some papers, it is suggested that during mechanical tests the walls of blood vessels behave as isotropic structures (Weizsacker and Pinto, 1988; Dobrin, 1999). For this reason, the walls of blood vessels are often modelled as an isotropic material (Raghavan and Vorp, 2000; Geest *et al.*, 2006a; Heng *et al.*, 2008). It has been proved here that AAA walls subjected to uniaxial tension can be modelled as an isotropic material, which corroborates the hypotheses put forward by Raghavan *et al.* (1996), Kobielarz *et al.* (2004) and Witkiewicz *et al.* (2007).

In the literature, it is generally believed that shear stress is not a significant factor in the analysis of AAA wall stresses, even though *in vivo* AAA walls are subject to multiaxial stresses, including normal and shear stresses produced by the blood flowing through the vessel. There are three reasons for the negligence of shear stresses. Firstly, the shear component is insignificant in comparison with the normal component of the stress vector (Truijers *et al.*, 2007). Peattie *et al.* (2004) established that the shear stresses in AAA walls were below  $2 \cdot 10^{-6}$  MPa, whereas the peak principal stress is at least 5 or even 6 orders of magnitude higher (Raghavan *et al.*, 1996; Vorp, 2007; Kobielarz *et al.*, 2008). Secondly, clinical observations and structural studies indicate that most of AAA walls have no distinguishable inner layer whereby the AAA wall is devoid of a functional layer of endothelial cells sensitive to shear stress (Holmes *et al.*, 1995). Thirdly, the majority of AAAs contain mural thrombus which may shield the wall against the action of shear stresses generated by the flow of blood (Wang *et al.*, 2002), and act as a damper (Vorp *et al.*, 1996). The structural examinations carried out as part of the present research revealed atrophy or degeneration of the inner layer in most of the AAA wall preparations. Because of the lack of a properly developed inner layer, the walls of the abdominal aortic aneurysms were

devoid of a functional layer of endothelial cells. Moreover, in 75% of the cases, mural thrombus sticking to the inner surface of the aortic wall was found to be present, although the significance of mural thrombus is debatable (Hans *et al.*, 2005). The shear stress neglect assumption has been proven through the indirect quality analysis. In the authors' opinion, the insufficient number of studies evaluating shear stresses in the walls of AAAs is one of the limits to the development of constitutive models for the AAA. Therefore, more research is needed in this area.

The lack of experimental verification of the assumptions concerning AAA wall incompressibility and isotropy is the main constraint of most constitutive models. The verified assumptions presented here have been incorporated to the constitutive equation derived from a hyperelastic model proposed by Raghavan and Vorp (2000) based on the generalized neo-Hookean model. Moreover, similarly as in Yamada *et al.* (1994), large strains have been introduced into the equation. Thus, model (3.10) takes into account the theory of large strains, the experimentally verified assumption about the incompressibility and isotropy of AAA walls and the structurally justified neglect of shear stresses. The proposed constitutive equation well approximates the stress-strain characteristics. The analytically determined material constants ( $\alpha$  and  $\beta$ ) assume lower values than the ones calculated from the model by Raghavan and Vorp (2000), although in the case of coefficient  $\beta$ , the order of magnitude is the same. The constant  $\alpha$  obtained in the present study is at least two orders of magnitude lower. The reduction is not due to the introduction of Green's strains. The coefficient  $\alpha$  assumes equally low values when the stress-stretch ratio curves are approximated with the constitutive equation proposed by Raghavan and Vorp (2000). It indicates that the best-fit material parameters depend on the results obtained for individual populations, particularly when the abdominal aortic aneurysm is a dynamic pathological process caused by structural changes of different intensity in the load-bearing elements. This indicates that it is necessary to take the degree of structural changes within the walls of abdominal aortic aneurysms into account in the description of experimentally determined curves.

## 5. Conclusion

On the basis of experimental verification, an evidence that the walls of abdominal aortic aneurysms behave as an incompressible and isotropic materials under mechanical loads is presented. Through indirect validation by using histological techniques, it was proved that AAA can be modelled on the assumption of negligence of shear stresses. The experimental results obtained for a large group of preparations during uniaxial tension test were fitted by a hyperelastic constitutive equation based on the generalized neo-Hookean model with contribution of the theory of large strains, the experimentally verified assumptions about the incompressibility and isotropy of AAA walls and the structurally justified negligence of the shear stresses. The parameters of the constitutive equation strongly depend on the individual variation in the particular investigated populations.

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