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Interaction of Biomechanics with Extracellular Matrix Components in Abdominal Aortic Aneurysm Wall

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WHAT THIS PAPER ADDS

The present results demonstrate for the first time, that in AAA locally acting biomechanical loads (stress and strain) are compensated for by increased synthesis of collagen and proteoglycans with increased failure tension of the AAA wall. These findings confirm the presence of adaptive biological processes to maintain the mechanical stability of AAA wall.

Objective: Little is known about the interactions between extracellular matrix (ECM) proteins and locally acting mechanical conditions and material macroscopic properties in abdominal aortic aneurysm (AAA). In this study, ECM components were investigated with correlation to corresponding biomechanical properties and loads in aneurysmal arterial wall tissue.

Methods: Fifty-four tissue samples from 31 AAA patients (30♂; max. diameter D_{max} 5.98 ± 1.42 cm) were excised from the aneurysm sac. Samples were divided for corresponding immunohistological and mechanical analysis. Collagen I and III, total collagen, elastin, and proteoglycans were quantified by computational image analysis of histological staining. Pre-surgical CT data were used for 3D segmentation of the AAA and calculation of mechanical conditions by advanced finite element analysis. AAA wall stiffness and strength were assessed by repeated cyclical, sinusoidal and destructive tensile testing.

Results: Amounts of collagen I, III, and total collagen were increased with higher local wall stress ($p = .002$, .017, .030, respectively) and strain ($p = .002$, .012, .020, respectively). AAA wall failure tension exhibited a positive correlation with collagen I, total collagen, and proteoglycans ($p = .037$, .038, .022, respectively). α-Stiffness correlated with collagen I, III, and total collagen ($p = .011$, .038, and .008), while β-stiffness correlated only with proteoglycans ($p = .028$). In contrast, increased thrombus thickness was associated with decreased collagen I, III, and total collagen ($p = .003$, .020, .015, respectively), and AAA diameter was negatively associated with elastin ($p = .006$).

Conclusions: The present results indicate that in AAA, increased locally acting biomechanical conditions (stress and strain) involve increased synthesis of collagen and proteoglycans with increased failure tension. These findings confirm the presence of adaptive biological processes to maintain the mechanical stability of AAA wall.

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INTRODUCTION

Interaction between biomechanical and biological factors in the pathogenesis of human abdominal aortic aneurysm (AAA) has often been postulated but not quantitatively demonstrated, and remains to be elucidated. It is hypothesized that out-of-balance vessel wall remodeling together with non-physiological mechanical conditions exerted by blood pressure, lead to progressive vessel expansion, aneurysm formation, and finally rupture.[1–4] Increased mechanical stress and strain in the vessel wall per se may induce biological alterations in ECM turnover and remodeling of the aortic wall. Thereby, not only degenerative but also compensatory changes in the vessel wall may be expected. For other cardiovascular disorders such processes have already been shown in vitro,[5] but at present not in the human blood vessel wall and in particular not in AAA.

Furthermore, material properties such as stiffness, strength, and failure tension of the load bearing vessel wall are determined predominantly by the quantity and functionality of various extracellular matrix (ECM) components

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Table 1. Baseline characteristics of patients.

Number of patients	31 (30 ♂, 1 ♀)
Age, years	70.1 ± 7.9 (range 60–80)
Max AAA diameter, cm	5.98 ± 1.42 (range 4.5–8)
Associated disease	*n*/%
Chronic kidney disease	6/20.7
Hypertension	19/62.1
Diabetes mellitus	6/20.7
Coronary heart disease	14/44.8
Smokers	22/72.4
Medication	%
ASA	69.0
Statins	48.3
ACE inhibitors	31.0
Beta blockers	62.1

such as collagen, elastin, and proteoglycans.[6–9] For AAA formation, an imbalance between degradation and synthesis of ECM components has already been described histopathologically.[8] However, for methodological reasons, neither the role of the acting mechanical conditions in ECM composition and remodeling, nor the impact of ECM composition on the material properties of AAA wall have been evaluated in vivo so far.[10–12] Still, not enough is known about the macroscopic mechanical behavior of the AAA wall in correlation with the composition of ECM within the vessel wall.[7] Moreover, at present it is speculated whether mechanical stress and strain are causative for adaptive or degenerative changes in the ECM composition of the AAA wall.

Meanwhile, the local mechanical loads acting on the AAA wall can be calculated realistically by finite element analysis (FEA), and the macroscopic mechanical properties of AAA wall can be measured experimentally.[13–17]

In this study, the acting mechanical stresses and strains were calculated, specific morphological AAA parameters assessed, and the local mechanical properties of the AAA wall were measured by means of tensile testing and analyzed in comparison with the corresponding and underlying local ECM composition.

METHODS

Study population and tissue sampling

Fifty-four tissue samples were collected from 31 AAA patients undergoing open surgical repair. Detailed patient characteristics are summarized in Table 1. Multislice computed tomography angiography (CTA) was performed in all patients. Using the commercial segmentation software Mimics (Materialise, Leuven, Belgium), CTA data were commuted for reconstruction of 3 dimensional (3D) AAA geometry including intraluminal thrombus for later finite element analyses. Moreover, 3D images were used to record the precise sample excision site and the orientation of the harvested AAA wall specimens during surgery, as described in detail in a previous study.[7] The tissue samples were then divided into two corresponding parts: one for biological analyses (approximately 15 × 3 mm) and one for mechanical testing (20 × 8 mm). The part for biological analysis was fixed with 4% formalin and embedded in paraffin. For mechanical analysis the specimens were stored in lactate Ringer's solution (130 mmol/L sodium chloride, 5 mmol/L potassium chloride, 2 mmol/L calcium chloride, 3 mmol/L sodium lactate) at 4 °C until the tensile tests were performed within 24 hours of surgery. Informed written consent was obtained from all patients. The study was approved by the ethics committee of the university hospital Klinikum Rechts der Isar, Technische Universität München, Germany.

Mechanical testing

The harvested AAA wall samples were cut into $n = 54$ individual rectangular specimens (typically about 20 mm × 8 mm) and subjected to uniaxial tensile testing as described previously.[7,14] Briefly, tissue samples were cleaned of all non-vessel wall components. Specimen thickness was averaged from five measuring points using a Mitutoyo "Quick-Mini Series 700" digital thickness gauge (Mitutoyo, Kawasaki, Japan). Elastic properties and failure loads were determined using a Bose Electro-Force 3100 tensile test machine (Bose Corporation, Eden Prairie, USA). First, the tissue samples were exposed to a cyclic sinusoidal test at frequency of 0.5 Hz with loading up to approximately 0.20 MPa (depending on specimen thickness). After applying 19 cycles of preconditioning, the data of the 20th cycle were used for evaluation.[7,11,14,17] Following the cyclic experiment, specimens underwent destructive testing to measure the failure load. In both cases, the applied force and the clamp displacement were recorded. Wall strength [N/mm^2] and failure tension [N/mm] were derived from destructive testing[7]:

$$strength = \frac{F_{max}}{A_0}, \quad failure\ tension = \frac{F_{max}}{specimen\ width}$$

with the maximum measured force F_{max} and the initial cross sectional area A_0 and width of each specimen. For a more detailed description of the experimental setup, the reader is referred to Reeps et al.[7]

The parameters α [N/mm^2] and β [N/mm^2] of the constitutive law utilized in the finite element stress and strain analysis were determined from the cyclic experiments using a Levenberg–Marquardt curve fitting algorithm.[7]

Finite element analysis

FEA was performed as described previously in detail.[7,14] Finite element models of the 3D AAA reconstructions were created and the corresponding patient specific nonlinear quasi-static structural finite element simulations were carried out using an in house research code BACI.[36] A hyperelastic constitutive law was used for the AAA wall based on strain energy functions proposed by Raghavan and Vorp[10]:

$$\Psi = \alpha(tr(C) - 3) + \beta(tr(C) - 3)^2$$

where $tr(C)$ denotes the trace of the right Cauchy-Green deformation tensor as a measure of strain. The two parameters α and β can be interpreted as stiffness in the small strain and the large strain regime, respectively. For the intraluminal thrombus (ILT) the constitutive model proposed by Gasser[18]