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Structural Differences in Collagen Morphologies between Healthy and AAA Tissues

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Introduction Arterial disease and degeneration can be linked to mechanical forces and alterations of the underlying mechanical tissue properties [1]. More specifically, changes in the structural components play an important role in the pathogenesis of aortic degeneration, e.g., in aneurysmal development [2]. The biomechanical behavior of healthy aortic walls is mainly determined by the activation of smooth muscle cells in the media and the elastin and collagen in the media and the adventitia. It is collagen fibers that give the aortic wall its required strength to be able to resist higher loads [3]. Experimentally obtained structural collagen data from healthy human tissues are becoming increasingly available, providing the relevant tissue parameters which can be utilized in numerical modeling using nonlinear structurally-based constitutive models [4,5]. It is essential that an accurate representation of the aortic wall structure be known, otherwise the predicted stresses through numerical modeling might not reflect 'true' *in vivo* conditions. We systematically compared healthy human abdominal aortic tissues with thrombus-covered tissues obtained from abdominal aortic aneurysms (AAA) to investigate differences between both tissue types.

Material and methods For the healthy tissue samples we used human abdominal aortas without atherosclerotic changes of type IV or higher that were harvested within 24 h of death. AAA tissue samples were provided to us immediately after surgical intervention of patients suffering from a risk of AAA rupture. All samples were cleaned of surrounding tissue (if necessary), cut into rectangular-shaped specimens with the longer side corresponding to the axial direction of the vessel, and subsequently fixed in 4% paraformaldehyde (PFA) overnight at room temperature. Next, excess PFA was rinsed off using phosphate-buffered saline (pH 7.4), and subsequently dehydrated using a graded ethanol series with each step lasting 45 min (once at 50 and 70, and twice at 95 and 100%). For optical clearing, we used a solution of 1:2 benyzl alcohol to benzyl benzoate (BABB) for 4 h before submerging all samples into a 100% BABB solution for clearing for at least 12 h [5].

Second-harmonic generation (SHG) imaging of collagen was performed using an imaging set-up consisting of a picosecond laser source and an optical parametric oscillator (OPO; picoEmerald; APE, Germany; HighQ Laser, Austria) integrated into a Leica SP5 confocal microscope (Leica Microsystems, Inc., Austria). Image acquisition was performed using a Leica HCX IRAPO L 25x0.95 water objective with a working distance of 2.5 mm for deep tissue imaging. Morphological collagen data was extracted and quantified from SHG images by combining Fourier power spectrum analysis with wedge filtering according to [6], yielding discrete angular distributions of relative amplitudes corresponding to the fiber orientations of the corresponding input image. Out-of-plane collagen fiber orientations were fitted using a π-periodic von Mises distribution [5-7] according to

$$\rho(\Theta) = \frac{\exp[b\,\cos(2(\Theta-\mu))]}{I_0(b)}, \tag{1}$$

where the fitting parameters are the concentration parameter b determining the shape of the von Mises distribution, and the location parameter μ describing the preferred (or principal) orientation of the fiber family; $I_0(b)$ denotes the modified Bessel function of the first kind of order zero, given by

$$I_0(b) = \frac{1}{\pi}\int_0^{\pi} \exp(b\cos\alpha)\mathrm{d}\alpha. \tag{2}$$

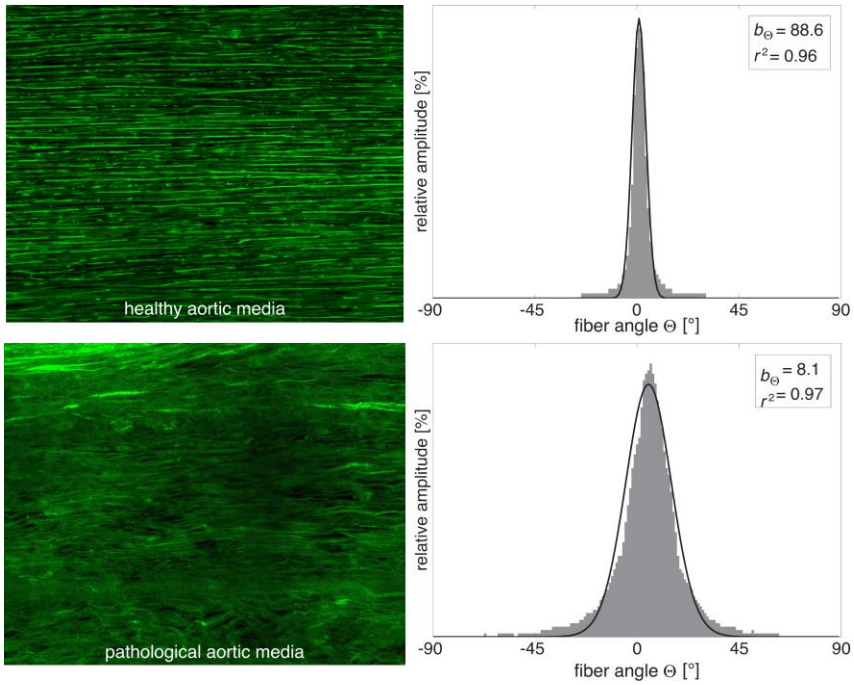

Figure 1. Transmural SHG images featuring collagen fibers (bright green) in the media of a healthy (top) and AAA arterial wall (bottom). The corresponding angular fiber distributions next to the images highlight the observed increase in the out-of-plane fiber orientations in AAA wall samples.

Results The healthy and diseased samples showed *remarkable* differences in the histological composition of the individual aortic layers, as well as in the out-of-plane collagen fiber arrangement. In general, within the AAA wall, the intima and media could hardly be distinguished (or dissected) from each other, and often the entire thickness of the wall including the adventitia had lost its characteristic structure of three individual layers, separated by elastic laminae. The out-of-plane fiber orientations differed significantly as illustrated in Fig. 1 (also in areas with no plaque or lipid pools present), where a much higher deviation from the in-plane fiber arrangement of healthy arterial tissue was observed in AAA samples (a more than tenfold increase of b).

Discussion Our results highlight the need to extend existing structural continuum frameworks to incorporate the observed structural differences between healthy and diseased arterial tissues. Otherwise, numerical results for AAA tissue (often based on structural parameters from healthy tissue) might not be a good predictor of the actual *in vivo* stress state. We suggest using a bivariate von Mises distribution $\rho(\Theta,\Phi) = N_c\rho(\Theta)\rho(\Phi)$ which is not rotationally symmetric and, therefore, considers the dispersion in both the azimuthal angle Φ and the elevation angle Θ; N_c denotes a normalization constant.

The use of human autopsy material was approved by the Ethics Committee of the Medical University Graz (no. 21-288 ex 09/10).

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