BIAXIAL STRETCH CAN HELP IN CORRECT INTERPRETATION OF COLLAGEN FIBRE ORIENTATION HISTOGRAMS

Michaela Turčanová (1), Jiří Fischer (1), Markéta Hermanová (2), Zdeněk Bednařík (2), Jiří Burša (1)

1. Institute of Solid Mechanics, Mechatronics and Biomechanics, Brno University of Technology, Czech Republic; 2. 1st Department of Pathology, St. Anne's University Hospital Brno, Czech Republic

Introduction

Structure-based constitutive models of arterial wall are important in biomechanical analyses. The most common models assume two symmetric fibre families, either perfectly aligned or dispersed [1], [2]. This assumption is seldom confirmed by histological analyses and the bimodal distribution of fibres may come from fibre waviness and from ignoring the differences between local and global orientations. This study applies automated polarized light microscopy for detecting the collagen fibre directions in porcine aorta under different types of biaxial extension eliminating the effect of waviness.

Methods

Porcine aortas were harvested in local slaughterhouse from 10 months old pigs. Specimens were cleaned and circumferential and axial directions were marked. Using a custom made biaxial tension device, they were fixed (under different biaxial stretches between 0.98 and 1.32) in 10 % formaldehyde solution at room temperature for 24 hours, then they were dehydrated and embedded in paraffin. Samples were sliced with microtome (5 μm thick) in the circumferential-axial plane and every slice was stained with 0.1 % Picro Sirius Red. The local orientation of collagen fibres was investigated in the unloaded state and under different levels of biaxial extension. If both stretches differed by less than 0.02, the extension was considered as equibiaxial.

Polarized light microscopy with an automatic algorithm [3] was used for evaluation of collagen fibre directions in up to 8 histological slices throughout the wall thickness (in ~10^5 points per slice). The resulting histograms were fitted with unimodal or bimodal von Mises distributions and grouped for inner (TM) and outer (TA) layers. The fitting procedure was performed in Curve Fitting Toolbox in MATLAB.

For statistical significance tests (α=0.05) one-way ANOVA was used. The data normality was verified by Kolmogorov-Smirnov test before performing ANOVA. Both analyses were performed in Minitab 15.

Results

For unimodal distributions the resulting mean angles μ_{av} and concentration parameters b are presented in Table 1. While no impact of load on the mean angle was found, statistically significant differences in dispersion were found between the unloaded state and both circumferentially-dominated and equibiaxial loads, while only negligible differences occurred between these last two groups. A similar tendency of fibres aligned more circumferentially with increasing circumferential extension (including equibiaxial states) was found also for bimodal distributions.

Load	TM		TA		TA+TM
	μ_{av} ± *SD*	b_{av} ± *SD*	μ_{av} ± *SD*	b_{av} ± *SD*	b_{av} ± *SD*
Unloaded	0.4 ±21.4	1.2 ±0.97	-9.5 ±24.7	0.88 ±0.31	1.03 ±0.71
Circumf.-dominated	0.3 ±10.0	2.03 ±0.85	4.8 ±4.7	1.56 ±0.63	1.77 ±0.73
Axially-dominated	4.0 ±22.1	1.2 ±0.61	13.0 ±44.6	0.75 ±0.43	0.95 ±0.53
Equibiaxial	4.4 ±10.8	2.13 ±0.71	3.8 ±12.0	1.33 ±0.51	1.73 ±0.72

Table 1: Mean angles μ_{av} [°] (measured from the circumferential direction) and concentration parameters b [-] (with their standard deviations SD) in aortic wall and its layers under different types of biaxial load.

Conclusion

The presented histological analyses have shown dominantly circumferential collagen fibre orientation and an increasing alignment of their local orientations under circumferential extension of the specimen. The distribution was either unimodal of bimodal. It was shown that all of these distributions got closer to circumferential direction with increasing circumferential stretch. This suggests that the bi-modality of the analysed distributions may be related rather to fibre waviness than their dispersion. This hypothesis is further supported by the increase of fibre alignment even with equibiaxial deformation, that cannot induce any fibre rotation, as well as by nearly identical changes under equibiaxial and uniaxial extension. Structure-based constitutive models are defined by global fibre orientations and all our results show fibre alignment in circumferential direction with their increasing extension. Thus they dispute the existence of two fibre families as interpreted from bimodal distributions of local fibre orientations detected in some arteries; their bimodality is probably caused by fibre waviness.

References

1. Holzapfel G.A: et al., J Elast, 61 (2000) 1-48.
2. Gasser T.C. et al, J R Soc Interface, 3 (2006) 15-35.
3. Turčanová et al, Biomed Res Int (2021) 6879765.

Acknowledgements

This work was supported by Czech Science Foundation project No. 21-21935S.