Acta Biomaterialia 57 (2017) 342–351

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

A comprehensive study of layer-specific morphological changes in the microstructure of carotid arteries under uniaxial load

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ARTICLE INFO

Article history:
Received 11 October 2016
Received in revised form 20 February 2017
Accepted 19 April 2017
Available online 9 May 2017

Keywords:
Carotid artery
Microstructure
Adventitia
Media
Uniaxial load

ABSTRACT

The load bearing properties of large blood vessels are principally conferred by collagen and elastin networks and their microstructural organization plays an important role in the outcomes of various arterial pathologies. In particular, these fibrous networks are able to rearrange and reorient spatially during mechanical deformations. In this study, we investigate for the first time whether these well-known morphological rearrangements are the same across the whole thickness of blood vessels, and subsequently if the underlying mechanisms that govern these rearrangements can be predicted using affine kinematics. To this aim, we submitted rabbit carotid samples to uniaxial load in three distinct deformation directions, while recording live images of the 3D microstructure using multiphoton microscopy. Our results show that the observed realignment of collagen and elastin in the media layer, along with elastin of the adventitia layer, remained limited to small angles that can be predicted by affine kinematics. We show also that collagen bundles of fibers in the adventitia layer behaved in significantly different fashion. They showed a remarkable capacity to realign in the direction of the load, whatever the loading direction. Measured reorientation angles of the fibers were significantly higher than affine predictions. This remarkable property of collagen bundles in the adventitia was never observed before, it shows that the medium surrounding collagen in the adventitia undergoes complex deformations challenging traditional hyperelastic models based on mixture theories.

Statement of significance

The biomechanical properties of arteries are conferred by the rearrangement under load of the collagen and elastin fibers making up the arterial microstructure. Their kinematics under deformation is not yet characterized for all fiber networks. In this respect we have submitted samples of arterial tissue to uniaxial tension, simultaneously to confocal imaging of their microstructure. Our method allowed identifying for the first time the remarkable ability of adventitial collagen fibers to reorient in the direction of the load, achieving reorientation rotations that exceeded those predicted by affine kinematics, while all other networks followed the affine kinematics. Our results highlight new properties of the microstructure, which might play a role in the outcomes of vascular pathologies like aneurysms.

1. Introduction

Cardiovascular disorders are a significant public health issue affecting ageing populations globally and causing considerable public health expenses (31% of total mortality in 2012 – source: World Health Organization). In numerous cases, the disorder involves significant changes in the vascular mechanical properties, generating extensive studies about arterial biomechanics and mechanobiology. In this respect, a common approach to vascular biomechanics consists in submitting samples of arterial tissue to mechanical bench tests in order to characterize their macroscopic mechanical properties. Existing investigations

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http://dx.doi.org/10.1016/j.actbio.2017.04.033
1742-7061/

[4,37,14] consisted in applying a tensile loading on flat samples of arterial material, revealing in particular the material's ability to undergo large strains and a characteristic stiffening occurring above a given tensile stress. In order to capture this complex mechanical behavior and a potential anisotropy of the response, uniaxial tensile tests have been performed independently in the axial and circumferential directions [32,8], and separately to the different layers of the composite structure of the arterial wall [22,46]. In parallel to this macroscopic characterization of the mechanical properties, the microstructure of the vascular wall has been extensively analyzed by different microscopy techniques. In particular, scanning electron microscopy allowed a morphological analysis of the arterial microstructure at the micron scale [20,55,12,35]. As for dynamic 2nd harmonic microscopy, it enabled simultaneous image acquisition and optical analysis of collagen fiber orientation, by means of the collagen's optical property of second harmonic generation in the presence of an intense laser beam coupled to a polarizer [43,48,26]. Confocal microscopy and multiphoton microscopy enabled live imaging with independent emission signals corresponding to elastin and collagen [50] providing a 3D point of view on morphological characteristics of the vascular wall, and allowing the evaluation of volume fractions of the different components of the microstructure [31]. These techniques revealed the morphology of each concentric layer (intima, media, adventitia). Concerning the intima, it is composed of endothelial cells, oriented longitudinally [37]. As for the media, it is composed principally of circumferentially oriented smooth muscle cells and collagen fibers embedded in an elastin network [56,10,53,13,16]. Finally, the adventitia is composed of thick collagen bundles and of a net of helically oriented elastic fibers [6]. Recently, a special attention has been dedicated to further characterize collagen and elastin fibers, for instance by measuring the waviness of adventitial collagen [36], the fiber segment length, and the radially-connecting fiber density in the media [49,29]. Also, several studies started investigating the link between the arterial tissue's macroscopic mechanical response and the associated rearrangements of its microstructure, by coupling mechanical testing with live microscopy. The latter studies confirmed the load-bearing properties of collagen fibers and revealed a progressive morphological rearrangement under load, namely decrimping and reorientation of the collagen fibers in the direction of the load, as well as the subsequent stiffening of the material's response [48,45,18,54]. Those tests consisted in the application of uniaxial tension on flat samples [52,21,38], biaxial tension on flat samples [39,23,47,27,28,7], or tension-inflation on cylindrical samples [19,58,17]. The observed morphological rearrangements concerned in particular bundle waviness and orientation [6,40,28,54].

As a conclusion, extensive characterizations of both the mechanical layer-specific anisotropic behavior of the arterial wall and the load-free microstructure morphology exist. Moreover, studies aiming at the characterization of load-induced microstructure rearrangements also exist and come up with advanced insights into the coupling between macro-mechanical response and tissue microstructure rearrangements. However neither the underlying microscopic mechanisms governing the load-induced microstructure rearrangements nor the inter-layer differences in the rearrangements of the collagen and elastin networks have been characterized. This paper is devoted to bridge these gaps in knowledge, by answering the following questions: are the well-known morphological changes of the vascular tissue under load (uncrimping, realignment) dependent on the direction of the load, on the vascular layer, and on the constituent under consideration? Are these morphological changes governed exclusively by the rule of affine transformations [5,25]?.

2. Materials and methods

2.1. Sample preparation

Seven carotid arteries (Fig. 1(a)) were harvested from healthy male New Zealand White rabbits, weighing 3 kg approximately. Excisions were realized at the Veterinary Campus of the Université de Lyon (VetAgro Sup, Marcy l'Étoile, FR). Rabbit cadavers, previously sacrificed under compliance with the NIH Guide for Care and Use of Laboratory Animals, were kindly provided by Centre Lago (Vonnas, FR). The length of each carotid was measured *in vivo* and *ex vivo*, i.e. immediately after harvesting (Table 1 – columns 2 and 3), in order to evaluate the *in vivo* pre-stretch condition (computed as the ratio of the *ex vivo* length to the *in vivo* length, Table 1 – column 4). The arteries were immediately frozen at −20 °C until the day of the experimental tests and unfrozen in a bath of phosphate-buffered saline (10x PBS, pH 7.1) at ambient temperature (24 °C). 10 mm long cylindrical portions were excised from the arteries and longitudinally cut open, with a resulting width of approximately 5 mm. For each artery, a 0.5 mm long ring (Fig. 1(b)) was also extracted for optical measurement of the arterial thickness (Table 1 – last column). This resulted in a cross-sectional area of 0.5 ± 0.1 mm^2. The rectangular strips were cut into dogbone shapes [21] aligned along the three following in-plane directions: circumferential, longitudinal, and an intermediate direction making a 45° angle with respect to the longitudinal direction (see Fig. 2(a) for a sketch of the sample preparation). In the following, this intermediate direction will be referred to as the diagonal direction. Twelve samples (four in each orientation group) were dedicated to mechanical testing coupled to multiphoton microscopy, while 6 additional samples (two in each orientation group) were dedicated to mechanical testing alone.

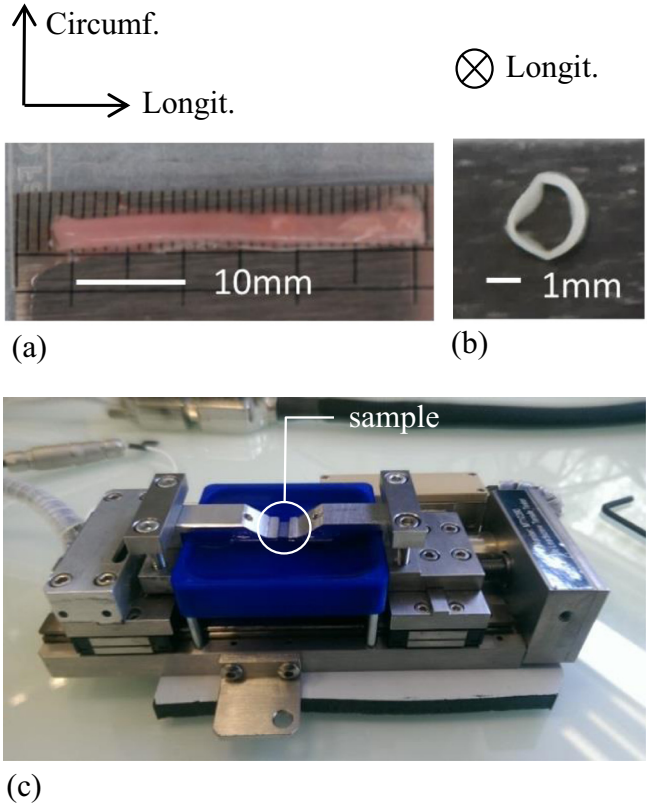

Fig. 1. (a) Excised carotid artery from a New Zealand White rabbit; (b) Cross-sectional ring of the artery for optical thickness measurement; (c) Tensile machine.