Review of the Techniques Used for Investigating the Role Elastin and Collagen Play in Arterial Wall Mechanics

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(Methodological Review)

***Abstract*—The arterial wall is characterised by a complex microstructure that impacts the mechanical properties of the vascular tissue. The main components consist of collagen and elastin fibres, proteoglycans, Vascular Smooth Muscle Cells (VSMCs) and ground matrix. While VSMCs play a key role in the active mechanical response of arteries, collagen and elastin determine the passive mechanics. Several experimental methods have been designed to investigate the role of these structural proteins in determining the passive mechanics of the arterial wall. Microscopy imaging of load-free or fixed samples provides useful information on the structure-function coupling of the vascular tissue, and mechanical testing provides information on the mechanical role of collagen and elastin networks. However, when these techniques are used separately, they fail to provide a full picture of the arterial micromechanics. More recently, advances in imaging techniques have allowed combining both methods, thus dynamically imaging the sample while loaded in a pseudo-physiological way, and overcoming the limitation of using either of the two methods separately. The present review aims at describing the techniques currently available to researchers for the investigation of the arterial wall micromechanics. This review also aims to elucidate the current understanding of arterial mechanics and identify some research gaps.**

***Index Terms*—Arterial mechanics, arterial microstructure, arterial stiffness, collagen, elastin.**

I. Introduction

CARDIOVASCULAR diseases are the leading cause of death in the world [1]. In 2014, the total number of deaths related to cardiovascular diseases was 778,000 in the United States alone, representing ∼30% of the total number of deaths [2]. Similar statistical data were estimated in England and Wales in 2013, with cardiovascular diseases being the second leading cause of death (28%) when considering all cancers together (29%) [3]. These data clearly point to the urgent need in identifying effective solutions to reduce the mortality of cardiovascular pathologies such as aneurysms, hypertension, atherosclerosis, and stroke. Most of the deaths related to cardiovascular diseases are classified as heart pathologies (∼614,000 in the USA in 2014), but arteries, determining the impedance against which the heart pumps, play a key role in the cardiac risk factor. For this reason, understanding arterial function and how it is impaired with pathologies is of crucial importance to reduce mortality. It is commonly accepted that arterial function is strictly correlated to the arterial wall internal structure; therefore, understanding the role of the arterial wall components and its micromechanics has been and still is a fundamental goal for research.

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A. Collagen and Elastin

Collagen and elastin are two major constituents of the arterial wall. Indeed, they contribute to 50-75% of the total dry weight of the arterial wall, which corresponds to 15-22% of the total hydrated weight. [4], [5]. Moreover, they are the major passive mechanical components of soft tissues; their molecular structure is consequently fundamental in characterising the mechanical behaviour of tissues.

Elastin is a protein that constitutes 90% of the elastic fibre. It is characterised by a very slow turnover (i.e. generation of new physiological tissues), and for this reason, the fragmentation that takes place in the elastin network due to ageing or pathologies has a dramatic chronic effect on the properties of tissues. Alternative splicing of RNA transcript allows the secretion of different forms of tissue function-specific tropoelastin. The crosslinking of tropoelastin by means of enzymes leads to the synthesis of the elastin core of the elastic fibre that also includes proteoglycans [6]–[9]. Elastin presents a helical structure at the microscopic levels, and this is thought to be one of the reasons behind its elastic properties [10]. At a molecular level, plausible mechanisms explaining elastin elasticity are entropy changes providing elastic recoiling force as seen in the classical theory of rubber elasticity and the highly dynamic behaviour of the hydrophobic domain of elastin [11].

Collagen, on the other hand, defines a wide family of proteins, characterised by repeating -Gly-X-Y- sequences and a triple right-handed helical structure constituted by three left-handed

helical polypeptide chains. Collagen types differ for their structures and consequently for their biological function. In the arterial wall, three types of collagen are present: type I and III, belonging to the fibril sub-family, and type IV, that forms network-like structures. The degree of collagen crosslinking is a key determinant of the mechanical properties of collagenous tissues, and its alteration dramatically changes arterial behaviour in pathology and ageing [7].

Collagen and elastin present significantly different mechanical properties which reflect the different functions they have in the arterial wall [12]. The modulus of elasticity of collagen is approximately 400 times greater than that of elastin: 100-130 MPa and 200-500 kPa, respectively [4], [10], [13]. It is commonly accepted [10], [14], [15] that, given their elastic moduli, collagen and elastin play different mechanical roles for the arterial wall, with elastin providing compliance, and collagen limiting the wall stretch and preventing mechanical rupture of the tissue. While the intrinsic mechanical properties of these structural proteins are important in determining the wall mechanical properties, their spatial arrangements are also crucial; indeed, both isolated collagen and elastin fibres maximum strain is lower than that of the arterial tissue *in-vivo* [14].

B. Arterial Structure

The arterial wall presents a three-layered structure: the innermost layer is the *intima*, the middle is the *media*, and the outermost is the *adventitia.* Each layer is characterised by different structural features and a different composition that is strictly related to its function. Therefore, given that different arteries play different roles in the cardiovascular system, the composition, structure and relative dimension of the arterial layers vary in different locations in the arterial tree [16]–[18]. The description provided in this paper refers to large arteries, typically the aorta.

The *intima* is composed of two sublayers; the innermost one is composed mainly of Endothelial Cells (ECs), a thin basement membrane, a proteoglycan-rich matrix, and few collagen fibres [19]. It constitutes the lumen and thus has the function to directly interface with blood, but it plays a negligible role in determining the elastic properties of the arterial wall [10]. The outermost sublayer is composed mainly of elastin fibres and individual Vascular Smooth Muscle Cells (VSMCs) [19]–[21]. Experimental results have proven that, at least in healthy arteries, due to its limited thickness, the *intima* has a negligible effect on arterial wall mechanics [22].

The *media* is highly rich in elastin and organised in concentric structural units, namely Medial Lamellar Units (MLUs) that repeat through its whole thickness (Figure 1). Each MLU is composed of an elastic lamella and an inter-lamellar space. The elastic lamellae are composed of circumferentially oriented elastin (71% of the total medial elastin), present small fenestrations in the elastin regular sheet-like structure [19], [23], [24], and show an undulated pattern in both the circumferential and longitudinal direction in the unloaded condition [15], [25], [26]. Elastic lamellae develop mainly in the circumferential direction but show branching and radial connections between adjacent MLUs [27], [28]. Fenestrations in the elastic lamellae allow

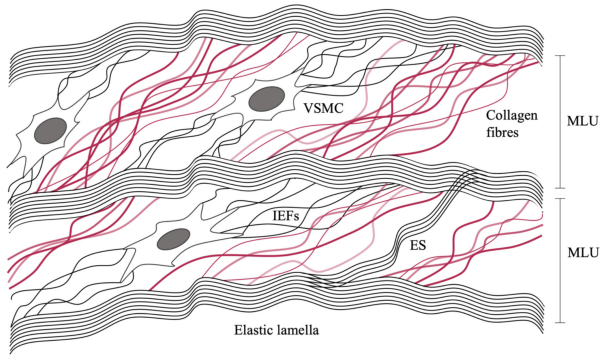

Fig. 1. Schematic cross-sectional representation of the arterial media. Medial Lamellar Units (MLU) are composed by the alternation of the sheet-like elastic lamellae and inter-lamellar spaces. The inter-lamellar space is characterised by a complex structure of Vascular Smooth Muscle Cells (VSMCs), collagen fibres, Elastin Struts (ES), and Inter-lamellar Elastin Fibres (IEFs).

cell signalling between different MLUs [23]. The internal and external elastic laminae (IEL and EEL, respectively) separate the *media* from the *intima* and *adventitia*, respectively. These elastin structures present larger fenestration than the elastic lamellae, thus a less regular shape [24]. On the other hand, the inter-lamellar space has a complex internal structure made by two families of elastin fibres, VSMCs, collagen, and mucopolysaccharide gel. In particular, inter-lamellar elastin fibres (IEFs) (27% of the total elastin content) form a connection between the elastic lamellae and the VSMCs [19], [29], having a tilt angle with respect to the circumferential direction around 19 degrees (circumferential-radial plane) and a circumferential orientation in the longitudinal-circumferential plane [29]. VSMCs are characterized by elongated elliptic nuclei sharing the same tilt angle of IEFs and seem to interact with both collagen fibrils and elastin lamellae [19], [29], [30]. Elastin struts (ES) (2% of the total elastin content) form a connection between adjacent elastin lamellae. VSMCs are surrounded by wavy collagen fibres that represent the principal component, in terms of volumetric fraction, of the inter-lamellar space [19], [28], [31]. Taking the circumferential direction as (0°) and the longitudinal as (90°), collagen orientation changes through the thickness of the media with an almost completely circumferential direction in the inner media and a $\sim$10° orientation in the outer media [32]. Also, elastin orientation changes through the *media* thickness, being circumferential in the central portion and assuming a more longitudinal orientation near IEL and especially the EEL [20], [33].

The *adventitia* is the outermost layer of the arterial wall, is relatively acellular and mainly constituted by collagen [34]. The internal part of *adventitia* (30-40% of its thickness) has a layered structure and is characterised by a similar volume fraction of collagen and elastin ($\sim$30%). The outer part of the *adventitia* has a higher content of collagen ($\sim$35%) and a lower content of elastin ($\sim$20%). Adventitial collagen is organised in crimped bundles of fibres in the unloaded artery, and its waviness is frequently quantified as the ratio between the fibre endpoint distance and the fibre length (approx. 0.8 in the unloaded configuration) [34]. The orientation of fibres in the arterial *adventitia* has been investigated using different microscopy techniques, such as

polarised light microscopy [35], [36], Diffusion Tensor Imaging (DTI) [37], [38], and Confocal Laser Scanning Microscopy [34], [39], [40]. However, the wavy structure of collagen fibres complicates the angle estimation. A wider angular distribution of collagen fibres was reported in the *adventitia* compared to the *media.* In fact, both longitudinally (0°), circumferentially (90°), and diagonally (±35-45) running collagen fibres have been detected in animal models [34], [38]–[41] and in human models [42]. In the inner *adventitia,* each layer is characterised by a single preferential orientation of collagen fibres, while elastin is organised in two families of fibres: the first sharing the same orientation as collagen fibres, and a second principal orientation. Therefore, elastin present a broader orientation spectrum than collagen [40], [43]. A change in the directionality of collagen fibres through the thickness of the *adventitia* has been identified by several authors; circumferential-diagonal in the inner portion, and longitudinal with a higher spread in the angular distribution towards the outer part of the artery [32], [41].

C. The Role of Collagen and Elastin in Arterial Wall Mechanics

The mechanical behaviour of arteries is generally classified into passive and active response. Passive response refers to arterial components whose behaviour cannot be modulated (i.e. the extracellular matrix constituted mainly of proteins). Conversely, active mechanics refers to the behaviour conferred to the arterial wall by active components (i.e. VSMCs) that affect the vascular tone through their activation. It is commonly accepted that collagen and elastin are the major determinants of the passive mechanical behaviour of arteries [4], [10], [14]. In fact, they constitute a large portion of the dry weight of arteries, and other wall components, such as VSMCs, have negligible values of passive stiffness compared to those of collagen and elastin [4], [10], [15]. However, the contraction of VSMCs can significantly alter the stress distribution across the wall and, therefore, the distribution of stresses between collagen and elastin, so that, *in-vivo*, passive and active responses are strongly interrelated [44]. Nevertheless, understanding the role of collagen and elastin on the passive mechanical properties of the arterial wall is of great interest as it provides a solid basis for studying more complex active mechanisms characterising the arterial wall *in-vivo*.

In a review on arterial mechanics describing the different roles that elastin and collagen play in arterial function, Burton [10] states "elastic fibres, with their great range of extensibility before elastic limit is reached, have the function of producing maintenance against the normal blood pressure fluctuation. The collagenous fibres, because of the architecture of the wall, are stretched only at higher than normal pressures and have a protective supporting role". This view is commonly accepted by other researchers [15]. The aim of this review is to identify the more relevant techniques reported in the literature to investigate the role of these structural proteins in the mechanical behaviour of arteries and the state of the knowledge on this scope. As already stated, the alteration of the arterial microstructure is essential to understand cardiovascular pathologies and consequent alteration of arterial function and mechanics. Several researchers have defined the pathologic remodelling process that happens in the arterial wall as an attempt to maintain homeostasis in terms of wall stress [45]–[48]. Since collagen and elastin are the main structural constituent of arteries, they play a crucial role in stress distribution through the wall thickness, and the remodelling process inevitably alters their organisation and content in the arterial wall. For this reason, it is crucial to identify solid methods to study the structure-related arterial mechanics.

II. Arterial Wall Mechanics

The arterial wall has been defined as "an incompressible nonlinearly elastic orthotropic material subjected to finite deformation" [49]. The first major implication of this definition is that stresses and strains must be defined with reference to the stress-free state [49]. Until 1983 the stress-free configuration had been identified as the no-load case; the configuration in which both the internal pressure and the axial stretch are released. In 1983, two different studies introduced new concepts on the behaviour of the arterial wall [50], [51]; the authors observed that, when cut radially, an arterial ring opens and assumes the shape of a circular arch. The cut-open configuration was identified as the stress-free configuration. This finding implies that the arterial wall in the unloaded configuration (zero internal pressure and no axial stretch) present residual strains and stresses due to the deformation, or closing, of the open circular arch in the arterial ring [52]. Several studies have proven that the physiological residual stresses are crucial in guaranteeing an almost uniform distribution of stress through the thickness of the arterial wall [52]–[54]. Blood pressure generates circumferential tensile stress that has its maximum at the inner radius and decreases monotonically towards the external radius. Oppositely, residual stresses are compressive in the inner portion of the arterial wall and tensile on the external one, compensating the stress gradient and guaranteeing a homogeneous distribution of stresses [45]–[48], [54].

The cut-open configuration is quantitatively described through the opening angle (OA), which is the angle between the two ends with reference to Greenwald [49]. The angle is defined by the extremes of the radially cut vessel ring and the middle point of the circular arch. While more recent experimental results have shown that residual stresses are still present in the cut-open configuration [49], [55], results in the porcine aorta showed that a multi-layered free-stress state definition does not introduce a significant change in the stress and strain estimation [56]. Recently, Holzapfel *et al.* [57] developed a complete layer-specific mathematical description of the residual stresses in arteries where not only the circumferential residual stresses but also the axial and radial ones were considered. Empirical results show the bending of the media in the axial direction, and constitutive modelling has demonstrated that this residual deformation furtherly homogenises the distribution of stresses in the arterial media [58].

The arterial wall can be considered as orthotropic because most arteries are subjected to negligible torsion *in-vivo*, thus expected to present very low values of shear strains. An exception can be found in the ascending aorta that, due to its proximity to the left ventricle, is subjected to higher and cyclical longitudinal stretches and twist along the vessel main axis

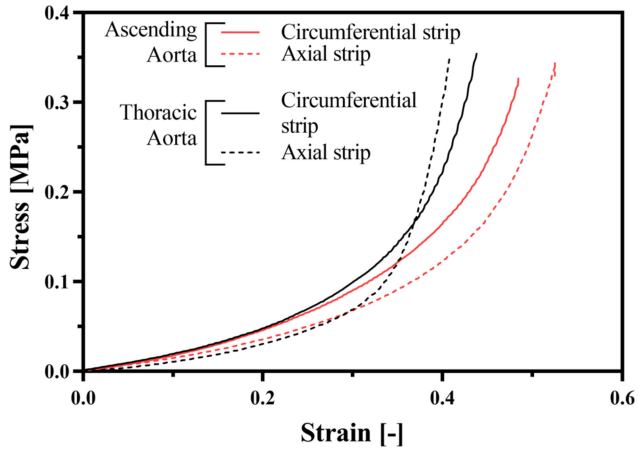

Fig. 2. The non-linear stress-strain relationship of the swine ascending (red lines as shown online; grey lines as shown in print) and thoracic aorta (black lines). Continuous and dashed lines indicate arterial strips tested in the circumferential and axial direction, respectively. At low levels of strain, the relationship is almost linear and elastin bears the entire load. At strain values of 0.40 and 0.25-0.30 for ascending and thoracic aorta, respectively, the stiffening process related to collagen recruiting starts. The last portion of the graph is dominated by the elastic behaviour of stiff collagen fibres. Moreover, the stress-strain relationship in the thoracic aorta (black lines) shows a stiffer response than in the ascending aorta (grey lines), thus showing the progressing increase in arteria stiffness moving from the heart to the periphery.

[59]. All relevant strains are oriented along the three axes of a cylindrical reference system centred on the vessel longitudinal axis [14]. Defining biological soft tissues as an elastic material is in principle incorrect; in fact, these tissues show softening when a cyclic load is applied, rate-dependent behaviour, and an area of hysteresis between the loading and unloading curves in the stress-strain relationship; a response that is so typical of a viscoelastic material. However, it has been observed that after a finite number of cycles, called preconditioning, the stress-strain relationships becomes repeatable, thus showing what has been defined as a pseudoelastic behaviour; meaning that two different elastic relationships can be identified through the loading and unloading curves [60]–[64]. Because the arterial wall experiences the highest stresses during systole of the cardiac cycle, the majority of studies concentrate on the loading curve after appropriate preconditioning.

The non-linear elastic relationship of the arterial wall (Figure 2) has been repeatedly demonstrated and is commonly accepted [4], [10], [26], [65]. The stress-strain relationship can be described as formed by two distinct portions connected by a transition region. At the onset of cardiac ejection *in-vivo*, or beginning applying load *in-vitro*, large strain variations are caused by small variations of stress, and that is why this first region of the stress-strain relationship persuasively indicates a low level of stiffness. At high levels of stress, instead, large changes in the stress level produce small strain variations, thus the artery exhibit high levels of stiffness. In the central transition region, a gradual stiffening is observed that graphically translates into an elbow in the stress-strain curve. A similar trend can be observed in the pressure-diameter curve. Since pressure and diameter are more evidently related to the vascular physiology than stresses and strains, often P-D graphs are preferred over stress-strain ones. Consequently, a set of indexes of the wall elasticity, such as distensibility ($Ds = \Delta V/[V\ \Delta P]$) and compliance ($Cs = \Delta V/\Delta P$), has been defined as a function of pressure and diameter [26], [66], [67] (where V is volume and P is pressure).

Arterial anisotropicity has been shown in a number of experimental works; in the physiological range of pressure, arteries show a higher stiffness in the circumferential direction than in the longitudinal one due to preferential fibres orientation (Figure 2) [64], [68], [69]. Also, the stress-strain curves show a higher dispersion in the longitudinal direction, indicating more variable mechanical properties. Since there is a strong relationship between structure and function in arteries, mechanical properties change significantly between different positions in the arterial tree; in general, a stiffening can be observed moving from proximal to distant regions [55], [70]. Moreover, considering a given location in the arterial tree, arterial mechanical properties, as well as geometrical features, change in different circumferential positions. For example, the posterior region of the pig aorta is the thinnest and stiffest, while the anterior is the thickest, but most compliant. These differences do not translate in different behaviour into terms of the pressure-diameter relationship since the stiffest region is also the thinnest, and vice-versa [71].

III. Investigating the Role of Collagen and Elastin in the Mechanical Response of Arteries

Collagen and elastin, being the main microstructural components of the arterial wall, are the main determinants of the passive mechanical behaviour of arteries. Several studies have tried to identify the role of these proteins on the mechanical response of vessels. The authors have identified four major methodologies used to achieve this goal: 1) microscopy 2) mechanical testing, 3) dynamic microscopy, and 4) mechanical testing and structure-based constitutive model formulation of the arterial wall mechanics. In the first case, arteries are fixed at different levels of transmural pressure and wall architecture is observed through microscopy techniques to obtain insight on the role of elastin and collagen on the wall mechanics. Mechanical testing, coupled with selective enzymatic digestion or wall composition analysis, represents a valid alternative to determine the contribution of collagen and elastin to the mechanical response of arteries. The third case represents a direct evolution of the first two, where mechanical testing and "live" microscopy are combined to obtain an almost continuous relation between mechanical behaviour and wall structure. Finally, structure-based constitutive models allow investigating the changes in model parameters between physiological and pathological conditions. Since these parameters are structurally motivated, their changes may provide valuable information on the alteration of the structural elements that they describe.

A. Microscopy

Microscopy, together with pure mechanical testing, is one of the oldest methods to correlate arterial mechanics to arterial

microstructure and can consist in the simple microscopic observation of the unstretched arterial wall or fixed at different transmural pressures. The structural changes that are observed at different steps of loading can then be correlated to the previously described mechanical behaviour of arteries. The range of microscopy techniques that have been used in this filed is quite wide: light microscopy [15], [35], [65], electron microscopy [15], [24], X-ray diffraction [71], Small Angle Light Scattering (SALS) [72], Optical Polarisation Tractography (OPT) [21], Confocal Laser Scanning Microscopy (CLSM) [34], [73], Scanning Acoustic Microscopy (SAM) [74], and Atomic Force Microscopy (AFM) [75]. In 1964, Wolinsky and Glagov performed a pioneering study on the structural reasons behind the static mechanical properties of the aortic media using light and electron microscopy. The abdominal aorta of New Zealand white rabbits was excised, cannulated in an *in-vitro* experimental set-up, and fixed at different levels of luminal pressure or axial stretch. It was observed that elastic *lamellae* present a wavy structure in both longitudinal and circumferential direction at 0 mmHg and no longitudinal stretch, as also confirmed in other studies [25], [73]. Lamellar waviness decreased in the direction of the applied stretch, while it remained unchanged in the other direction. Lamellar straightening was also accompanied by a decrease in inter-lamellar thickness. In the circumferential direction, the elastic *lamellae* showed straightening between 0 and 80-100 mmHg, while an almost constant structural organisation was observed in the range 100-200 mmHg. The inter-lamellar elastin fibrils network showed a low level of alignment at 0 mmHg. Increasing the intraluminal pressure, the degree of alignment increased, reaching a plateau at 80-100 mmHg. On the other hand, collagen fibres were present in the form of bundles with no consistent arrangement for pressures below 80 mmHg. When the pressure was raised to 100-150 mmHg, collagen showed a circumferential orientation, and it was organised in separated fibres uniformly distributed. Similar results were found by Sokolis *et al.* [65] when performing histology on arteries that were fixed at increasing levels of axial stretch. These results prove that the straightening and alignment process of elastin and collagen in the *media* takes place in two different ranges of pressure, namely from 0 to 80-100 mmHg and from 100 to 150 mmHg for elastin and collagen, respectively. This explains the biphasic stress-strain or pressure-diameter curves of arteries with elastin responsible for the first highly compliant portion of the curve and collagen causing the gradual stiffening at high levels of pressure. These findings confirm the widely accepted theory that elastin is the major mechanical component of the arterial wall at physiological pressures, while collagen protects the wall in case of high non-physiological level of pressure.

Light microscopy, coupled with fractal analysis, has also been used to study the effect of ageing and fatigue on the artic medial elastin network of different animal species. It was shown that elastin is subjected to fatiguing and network alignment decreased with the number of heartbeats, thus affecting the stress distribution in the arterial wall and load-bearing of elastin and collagen [9].

Other microscopy techniques, such as X-ray diffraction, SALS, polarised light microscopy and, OPT, do not allow the direct visualisation of protein arrangement in the arterial wall but provide information on their orientation. A second major limitation of these methods consists in the incapability of discerning between collagen and elastin fibres, thus providing global information on fibres orientation in the wall. In an experimental study on the bovine carotid artery, Bigi *et al.* [71] fixed arterial rings at different levels of circumferential stretch and analysed fibres orientation *via* X-ray diffraction. An alignment towards the direction of the load was observed. Similar results were obtained using SALS; fibres present a preferential circumferential orientation in the *media*, while the *adventitia* also shows a second longitudinally oriented family. Increasing the level of circumferential stretch a higher level of alignment was found in the *media*, but it was not present in the *adventitia* [72]. Timmins *et al.* [20] used Multi-photon Microscopy to study the effect of biaxial mechanical testing on collagen and elastin orientation and alignment in a specific cross-over region of the inner media where fibres shifts from the axial to the circumferential direction when moving radially from the *intima* deep into the *media*. Interestingly, fibres alignment increased only when axial loading was applied. On the contrary, circumferential stretching produced a decrease in fibres alignment, possibly suggesting the reorientation of non-circumferentially oriented fibres towards the load direction. Canham *et al.* [35] analysed fibres orientation in human coronary arteries fixed at 120 mmHg using polarised light microscopy. While in the coronary *media,* the orientation information is mainly related to VSMCs, in the adventitia, it refers mainly to collagen. The authors reported a preferential circumferential direction of fibres in the adventitia but with a significant degree of dispersion towards the longitudinal direction. Moreover, the dispersion is also observed in the radial direction, and according to Canham *et al.* [35], this could be justified by a certain degree of fibre waviness. This observation gives a structural justification to the anisotropic behaviour of the arterial wall, which is stiffer in the circumferential direction, and prove that the stiffening phenomenon that is observed is due to structural protein unfolding and alignment in the load direction. Moreover, these findings seem to demonstrate that the *media* is the major determinant of the shape of the pressure-diameter curve of the arterial wall since no change in the alignment was shown in the *adventitia* by Gaul *et al.* [72] and collagen waviness was observed by Canham *et al.* at 120 mmHg. Given the fact that the *adventitia* is mainly collagenous while the *media* has a high elastin content, the theory on the different role of collagen and elastin in the arterial mechanics described by Burton [10] is furtherly confirmed.

SAM and AFM reside on the borderline between microscopy and mechanical testing, since providing both topological and mechanical information on the scanned substrate. SAM is a particular microscopy technique that exploits ultrahigh frequency acoustic waves to investigate the microscopic mechanical features of a specimen. Indeed, the topographical information is obtained by the wave speed that is dependent on the tissue stiffness. The sound frequency determines both the spatial resolution and the penetration depth of the stiffness mapping: the higher the frequency, the higher the spatial resolution and the lower the penetration depth. Graham *et al.* [74] studied the effect

of ageing on the mechanical properties of the ovine ascending aorta at a microstructural level using SAM. It was shown that the wave speed (stiffness) increased in old animals. Moreover, the stiffness mapping allowed localising *medial* structures such as elastic *lamellae* and VSMCs rich interlamellar region. The age-related stiffening was proven to be related to an increase in the collagen content in the inter-lamellar space. Again, collagen is proven to provide high stiffness to the arterial wall, and alteration in its physiological concentration in the media alter the mechanical behaviour of arteries significantly [74], [76]–[78].

AFM exploits the motion of a cantilever over the surface of the sample to derive information on topological and mechanical features at the nanoscale level [79]. A study on the internal mammary artery showed a significant correlation between high and low levels of regional Pulse Wave Velocity (PWV) and the stiffness, thickness, and D-period of adventitial collagen fibres [75].

B. Mechanical Testing

One of the simplest methods to investigate the role of collagen and elastin in the passive mechanical response of the arterial wall consists of comparing the stress-strain curves with the wall composition. Cox performed a series of studies to find a correlation between the mechanical properties of arteries and their collagen to elastin ratio [4], [80]–[82]. Interestingly, the collagen to elastin ratio was shown to be a good predictor of the mechanical response of arteries at a given location in the arterial tree (ageing and hypertension), but it was not when comparing different arterial sites. This means that the relative quantities of collagen and elastin are not sufficient to describe the arterial mechanical properties, and microstructural organisation, distribution through the wall thickness and different compositions of structural proteins are crucial for arterial mechanics. Further, using simple models of arterial mechanics where elastin and collagen act as springs in parallel, Cox provided a first estimation of the load-bearing percent of collagen fibres with increasing luminal pressure, and mathematically described the gradual disentanglement of collagen fibres with increasing circumferential strain [4]. Approaching the issue from a different perspective, Berry *et al.* [83] estimated the pressure-radius relationships the rat aorta would have if it was constituted only of elastin, exhibiting a constant elastic modulus independently of pressure. Comparing experimental results and modelled relationships, they showed that below 75 mmHg the pressure response of the artery is due to elastin alone.

As indicated above, when dealing with soft tissues, the definition of the stress-free configuration is of crucial importance. The unloaded arterial rings present residual strains that guarantee more uniform distribution of stresses in the physiological loading condition. The structural proteins that constitute the arterial wall and their geometrical arrangement are responsible for the existing of residual stresses and strains in the arterial wall, and, for this reason, their role has been investigated. Ageing and pathologies may alter proteins concentration and their structural organisation, thus producing an alteration of the stress distribution through the wall thickness that may result fatal in the case of aortic aneurysms. Vassoughi *et al.* [84] were the first to notice that a single radial cut was not sufficient to reveal the stress-free configuration of arteries, as performing a further circumferential cut produced two layers with different OAs, thus indicating that residual stresses are still present in the cut-open configuration. Greenwald *et al.* [49] further studied the distribution of residual stresses across the thickness of the arterial wall and found that exterior machining of the bovine carotid produced an increase in the opening angle. In contrast, interior machining removed elastic layers form the inner portion of the wall, thus decreasing the opening angle. Moreover, the effect of elastase treatment, collagenase treatment and VSMCs removal on the opening angle of the rat aorta was investigated: only elastase produced a significant reduction in the opening angle. This result was additionally confirmed by Fonck et al. [26]. The similarities between the effect of elastase treatment and interior machining led the authors to the conclusion that the latter caused a gradual removal of medial layers, which are rich in elastin, thus producing a similar effect to direct elastin fragmentation. Moreover, collagen has been described as wavy and relaxed in the unloaded configuration, so the independence of the opening angle from the collagenase treatment is structurally well motivated. Peña *et al.* [55] further clarified the distribution of the residual stresses through the thickness of the arterial wall. Arterial layers were physically separated to assess the layer-specific OAs. A higher OA was identified for the intima in both proximal and distal porcine aortic rings. Furthermore, Shahid *et al.* [85] studied the effect of the releasing of the residual stresses on the alignment of fibres in the media. In the intact vessel, a decrease in the alignment of fibres was observed with increasing radius. On the other hand, such a trend was not observed in cut open samples. A higher degree of fibres alignment in the inner media has been reported by several authors already cited in the previous paragraphs. When the sample is cut open, the residual stresses are released, thus is reasonable that a lower level of alignment is found through the whole wall.

As for the opening angle, several works have exploited collagenase and elastase treatment to study the contribution of collagen and elastin networks to the mechanical behaviour of the arterial wall. The efficacy and selectivity of these enzymatic treatments have also been proven [26], [64]. Clearly, the first attempt of these works is to understand the role of collagen and elastin in the non-linear stress-strain or pressure-diameter relationship of the arterial wall. In 1957, Roach and Burton were the first to use the selective digestion of elastin and collagen to explain the non-linear mechanical behaviour of the human iliac artery undergoing circumferential uniaxial testing, showing elastin determines the stress-strain behaviour at low pressure while collagen becomes dominant at high pressures [86]. In 1983, Dobrin *et al.* [87] conducted a similar study performing biaxial mechanical testing of the dog carotid artery and showed that elastase caused a change in the stress-diameter curve over the whole range of diameters, while collagenase caused a difference with respect to the untreated samples only for pressure levels above 60 mmHg. These results have been confirmed in several other papers [26], [64], [88] and suggest that at low pressure the load is borne entirely by elastin, while

collagen contributes to arterial mechanics only at high levels of arterial pressure. Similar results have been obtained when collagen was removed with autoclave treatment, and differences in elastin tissue stiffness were identified between aortic proximal and distal sites, with the latter being stiffer. Moreover, at high levels of pressure collagen becomes the major determinant of the stress-strain relationship, even though the percentage of load-bearing remains significantly lower with respect to elastin in the physiological range of pressure [89]. However, it is worth noting that, given the complex structural organisation of the arterial wall and the interaction between structural proteins and VSMCs, the removal of one component of the arterial wall affects the structural arrangements of the others, so that, for example, the behaviour of collagen in the intact wall and in the elastase treated wall might differ [90].

Relevant findings are also related to the anisotropicity of untreated and treated tissues. Elastase treated samples maintain the anisotropic mechanical behaviour of untreated arteries, while collagenase treated samples show an almost isotropic behaviour [64], [91]. These results suggest that the elastin network has an almost isotropic behaviour, while collagen is responsible for the anisotropic behaviour of the arterial wall. On the contrary, the biaxial experiment performed by Dobrin *et al*. [87] showed that the removal of elastin produced a constant decrease of 60% in the axial level of stress independently on the axial and circumferential level of load. On the other hand, the decrease of the circumferential stress level was dependent on both the increasing longitudinal and circumferential load levels. These findings seem to suggest an anisotropic behaviour of the elastin network, but Wolinksy *et al.* [15] showed that elastic lamellae are completely straight in the axial direction at the physiological level of longitudinal stretch (used in the biaxial test of Dobrin) and that complete straightening in the circumferential direction is reached only at 80 mmHg. For this reason, the stiffening of elastin in the circumferential direction reported by Dobrin *et al.* can be explained even assuming an isotropic behaviour for elastin as reported in different studies. The removal of collagen, instead, had no significant effect on the longitudinal level of stress, while it had a significant effect on the circumferential stress at high levels of pressure. According to this result, collagen does not respond to axial loading. On the contrary, Gundiah *et al*. [91] showed that collagen plays a role also in the axial direction. The mainly circumferential orientation of collagen fibres in the arterial wall seems to explain the results of Dobrin *et al.* [87]. However, microscopy images have proven the existence of longitudinally directed collagen fibres, especially in the adventitia, that may affect the wall mechanics in the longitudinal direction, as reported by Gundiah *et al.* [91]. Additional work has proved that elastin supports almost the entire compressive load also in the radial direction [92].

Similar results have been found using a different solution that consists in physically separate the three arterial layers and test them under uniaxial or biaxial stretch. A higher level of anisotropicity was detected in the *media* with respect to the *intima* and *adventitia* (Figure 3), especially in the proximal region of the porcine aorta. Moreover, all the layers showed a pronounced non-linearity, even though the stiffening in the *media* was more gradual than in the other two layers, and the level of stiffness was lower [22], [55]. While the higher linearity detected in the media is coherent with the high content of elastin in this layer, the high anisotropicity found in the media is structurally justified by the higher level of alignment of collagen fibres in this layer compared to the *adventitia*. Indeed, the stress-strain relationships of the *media* in the work of Peña *et al.* showed higher linearity in the longitudinal direction, along which few collagen fibres are aligned, than in the circumferential one. Interestingly, Butcher [21] reported also an increase in arterial stiffness with ageing, and it is commonly accepted that ageing is associated with fragmentation of elastin, increase of collagen crosslinking and content [7], [8], [74], [93]–[96]. Other studied have reported an increase in arterial stiffness and anisotropy with increasing age [94], [97], [98]. As expected, elastase treatment and ageing produce similar changes in the mechanical behaviour of the arterial wall since in both cases a more collagenous tissue is obtained. However, it is worth noting that the mechanisms behind the two processes differ significantly; while collagen concentration increases with ageing due to collagen synthesis, elastase produced only an increase in collagen relative quantity due to the removal of elastin. As mentioned above, removing elastin might also affect the structural organisation of other components of the extracellular matrix.

Finally, Weisbecker *et al.* [64] focused their study on the role of collagen and elastin on the viscoelastic behaviour of the arterial wall and observed that elastase treated samples showed softening with increasing number of load cycles, while collagenase treated samples did not exhibit any softening. These results suggest that elastin fibres show an almost perfect elastic behaviour independently on the presence of collagen fibres. In contrast, the collagen network seems to be dependent on the presence of elastin to maintain its microstructural organisation. Elastin and collagen networks are usually considered as acting in parallel [41], [99]–[101], but these findings indicate a more complex interaction between the two structural proteins. In agreement, residual deformations have been found in creep experiments on elastase treated samples, while load caused plastic deformation in untreated arteries and collagenase treated arteries did not differ significantly [102].

C. Dynamic Microscopy

From a structural point of view, arteries have been defined as a composite material consisting of fibres, elastin, and collagen, embedded in a compliant and viscoelastic matrix, made of ground substance and cells. As already stated, the matrix role in arterial mechanics is negligible with respect to collagen and elastin fibres. Therefore, to determine the properties of the composite material, it is sufficient to know the mechanical properties of the fibres, their spatial orientation, and concentration. The coupling of "live" microscopy and mechanical testing of the arterial wall allows studying the effect of changes in spatial orientation and concentration of Extracellular Matrix (ECM) proteins on arterial wall mechanics. The main evolution with respect to simple microscopy consists in the fact that mechanical testing

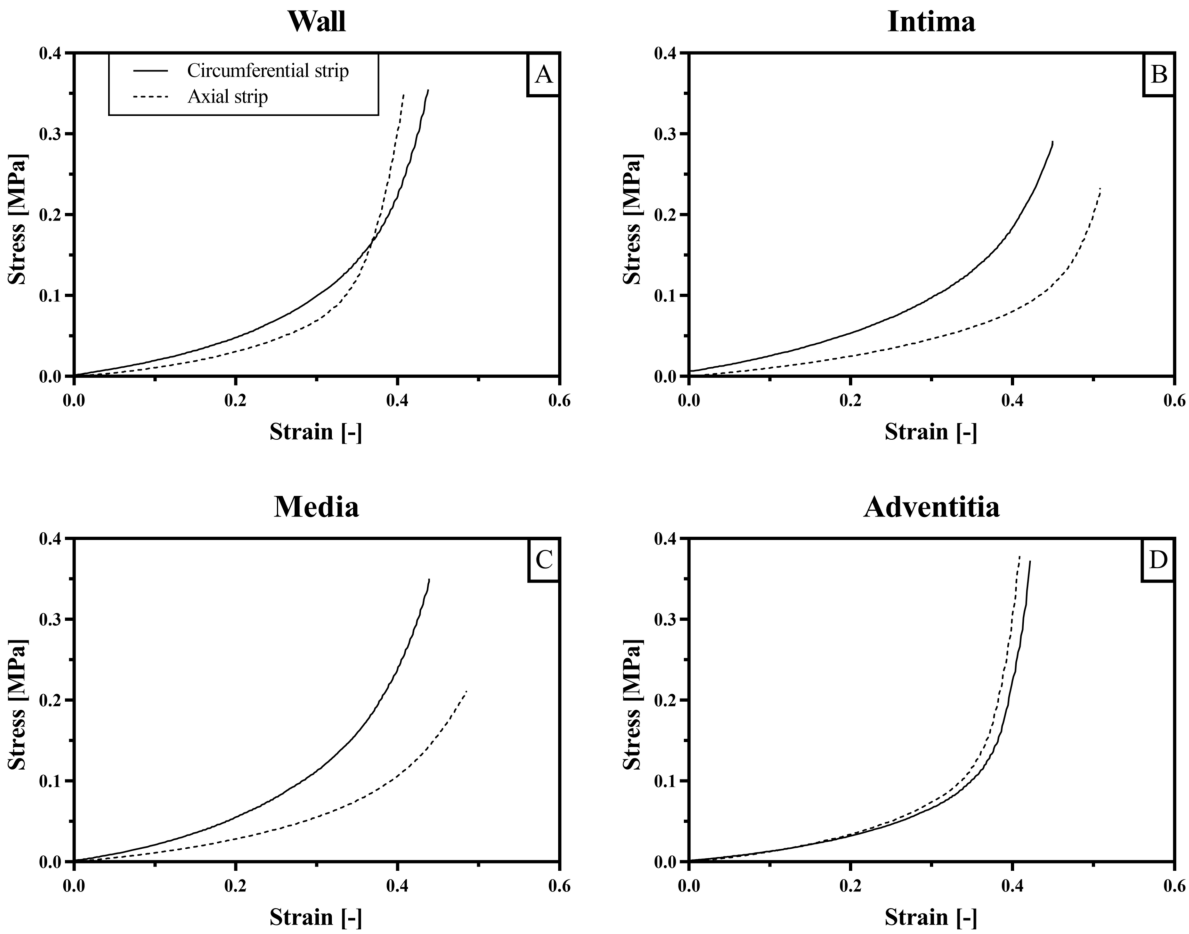

Fig. 3. Stress-strain relationships of the intact wall (3A), intima (3B), media (3C) and adventitia (3D) of a swine thoracic aorta subjected to uniaxial testing. Medial strips exhibit gradual stiffening with increasing strain level and marked anisotropic behaviour (3C). On the other hand, the highly collagenous adventitial tissue shows a clear non-linear and isotropic mechanical behaviour (3D). The mechanical behaviour of the intact wall combines the properties of the medial and adventitial layers: at low strain level and in the physiological pressure range (0.07–0.09 MPa), the arterial wall is stiffer in the circumferential than axial direction, reflecting the behaviour of the media. At high strain levels, the arterial wall is more isotropic due to the recruitment of collagen fibres in the adventitia. Due to the different composition and microstructure of the three arterial layers, mechanical testing of isolated arterial layers allows identifying the role collagen and elastin play in arterial mechanics.

and imaging are performed simultaneously, and the microscopy techniques are non-destructive. Therefore, an almost continuous structural-mechanical information can be obtained on the same artery with increasing load level. Multi-Photon Microscopy (MPM) is the most widely adopted microscopy technique to perform this type of study. This technique exploits non-linear imaging to visualise elastin (Two-Photon Fluorescence-TPF) and collagen fibres (Second Harmonic Generation-SHG) up to 200 μm deep in the arterial wall without the necessity of tissue staining. MPM can be combined with mechanical testing of different level of complexity. Uniaxial or ring tests are quite simple to perform but have a poor mimicking power of the *in-vivo* loading condition and fibres spatial arrangement is affected. Planar biaxial tensile devices are closer to the physiological loading condition, but the flattening of the arterial sample and the consequently introduced initial strains are still non-physiological. In inflation tests, the degree of complexity is furtherly increased, especially when a longitudinal stretch is applied simultaneously, but they are more physiologically motivated [103]. Moreover, the amount of tissue that has to be used in the last case is higher, and this may result in a problem when human arteries are tested.

Applying a uniaxial load in different directions on rabbit carotid artery strips, Krasny *et al.* [31] identified different levels of wall stiffness when circumferential, longitudinal and diagonal loads were applied, as well as different behaviours of collagen and elastin in both *media* and *adventitia.* More specifically, the highest stiffness was reported for the circumferential direction, while the most compliant was the longitudinal one. All the stress-strain relationships showed high non-linearity, independently on the direction of load. The first highly compliant part of the stress-strain curve observed in all the directions was structurally explained, as expected, by the increase in alignment in medial elastin observed independently of the loading direction. Moreover, a certain degree of realignment and uncrimping of collagen fibres in the media or adventitia was observed in all the investigated directions of application of the uniaxial load, justifying the stiffening that happens at high stretches for all the samples. MPM images showed a circumferentially oriented wavy collagen in the media, while a highly crimped longitudinally oriented collagen

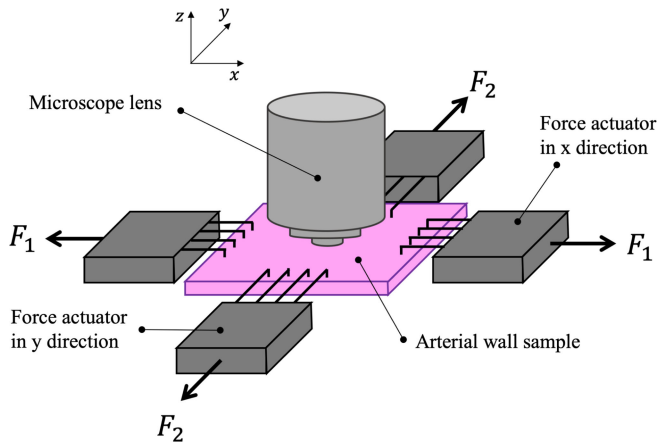

Fig. 4. Schematic representation of a typical set-up for dynamic microscopy under biaxial testing. The arterial wall sample is mounted on a device for tensile testing of relatively small dimensions in order to allow its positioning under a microscope lens. The sample is then stretched eccentrically in both the x and y direction so that the region of interest remains in a fixed position with respect to the lens. The force F_1 and F_2 can be controlled independently so that both equbiaxial and non-equibiaxial test can be performed.

was observed in the adventitia. A possible explanation for the different timing of the stiffening phase can be found in the different levels of waviness in the different arterial layers. Medial circumferentially oriented collagen fibres may be recruited sooner than adventitial longitudinally oriented collagen due to its lower degree of waviness. However, it is crucial to consider that the flattening of the arterial sample introduces tensile strains in the inner part of the artery, while compressive strains are caused in the external one. This may introduce artefacts in the crimping level of collagen in the media and adventitia. An alternative explanation for the anisotropic behaviour can be that adventitial collagen realigns along the loading direction in all the loading configurations. On the other hand, a longitudinal load increased the crimping of the medial collagen, while circumferential and diagonal ones induced uncrimping. Therefore, medial collagen seems to be ineffective in the longitudinal direction, justifying the lower arterial stiffness in this direction.

Chow *et al.* [88] performed equibiaxial and non-equibiaxial tensile tests on squared porcine thoracic aorta samples, coupled with simultaneous CLSM imaging. With equibiaxial strain, adventitial collagen showed a realignment from the circumferential direction towards the longitudinal one with increasing strain, while medial collagen aligned in the circumferential direction. In the case of a higher load in the circumferential direction, circumferential realignment was also observed in the adventitia. No significant change was observed in medial elastin in both cases. Also in this case, the straightening process of collagen in the adventitia was observed at higher strains than in the media, confirming that this difference might play a role in the anisotropic response of arteries. Again, the flattening of the sample might introduce artefacts in collagen waviness. Figure 4 shows a schematic representation of a typical biaxial load dynamic microscopy experiment.

Two works have been identified in which the arterial segment was subjected to pressurisation only (i.e. without axial stretch) [104], [105]. Sugita *et al.* [105] reported a higher level of crimping of collagen with respect to elastin in the mouse and rabbit media, in agreement with the subsequent recruitment of elastin and collagen at low and high pressure, respectively. Two families of circumferentially oriented collagen fibres where identified and they were associated with VSMCs and elastic lamellae, respectively. Elastic lamellae associated with collagen fibres showed a delayed uncrimping starting at 80-100 mmHg with respect to VSMCs related fibres (40 mmHg). These results point out that some collagen fibres seem to be recruited even at very low-pressure values, while those fibres running tangentially to elastic lamellae in the circumferential direction start the uncrimping process at physiological pressures and continue to straighten at supra-physiological pressures. Cavinato *et al.* performed membrane inflation test on porcine and human aortic samples to observe collagen alteration in the adventitia [42]. While fibres preferential diagonal orientation was unchanged, the orientation spectrum showed an increase in alignment at high levels of pressure. Moreover, collagen was shown to be still partially crimped at physiological levels of systolic pressure, and complete straightening was reached at 200 mmHg. While the physiological meaning of this experiment is controversial, it showed that adventitial collagen bears a minor portion of the load in all the physiological range of pressures.

Tension-inflation tests are the most suitable option to simulate the *in-vivo* arterial condition but require a higher level of complexity of the experimental procedure. Moreover, the limited penetration power of MPM (100-200 μm in the arterial tissue) limits the investigation to the adventitial structure only since the microscope lens can approach the sample only on the adventitial side. Chen *et al.* [40], [43] studied load-induced microstructural changes in the porcine coronary. It was showed that with increasing pressure (0-140 mmHg) collagen fibres tend to realign from a diagonal (approx. 60° with respect to the circumferential direction) direction towards the circumferential direction (approx. 40°), thus giving a possible explanation to the anisotropic behaviour of arteries. Adventitial elastin showed an even higher realignment towards the circumferential direction. Moreover, at the physiological stretch of 1.5 and 1.3 for the circumferential and longitudinal directions respectively most of the adventitial collagen fibres were still crimped, suggesting that the portion of load borne by collagen is still limited. Good correspondence was also found between collagen straightening at $\lambda_z = 1.5$ and $\lambda_\theta = 1.8$, and the stiffening in the longitudinal and circumferential stress-strain relationship. Similar results on the collagen orientation were obtained in other works on the rabbit carotid artery [39], [106]. Schriefl *et al.* [107] introduced a technique for the optical clearing of soft biological tissues to allow for the imaging of whole organs independently on their thickness. While overcoming the limitation of the low penetration power of MPM, this technique presents the major drawback of requiring the fixation of the tissue, so that dynamic imaging cannot be performed, and the tissue can only be imaged at the circumferential and axial stretch imposed during fixation.

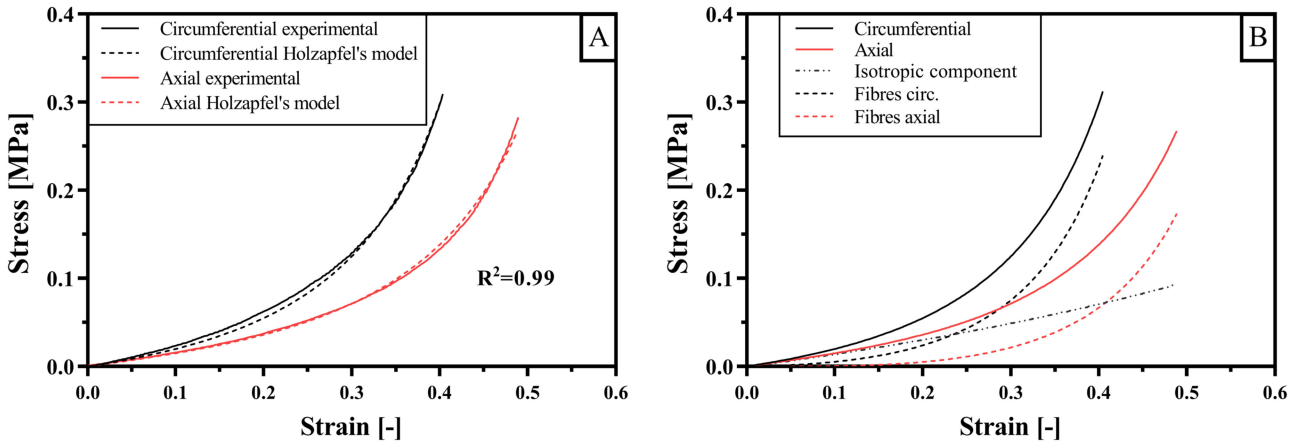

Fig. 5. Fitting of circumferential and axial uniaxial tensile test of the swine ascending aorta with the Holzapfel's strain energy function. Panel 5A, comparison between experimental and fitted stress-strain relationship in the circumferential and axial direction. Due to the stiffer behaviour in the circumferential direction, the model estimated the fibres angle at 32° with respect to the circumferential direction. Panel 5B, decomposition of the circumferential and axial stress-strain relationship in isotropic (coinciding in the two directions) and anisotropic (fibres) components. The fibrous component shows a stiffer mechanical response in the circumferential direction.

D. Structure-Based Constitutive Models

Different constitutive models have been formulated to describe the passive non-linear stress-strain relationship of the arterial tissue through strain energy functions (SEFs) [13], [108], [109], and two main schools of thought can be identified: polynomial [110] and exponential [63] SEFs. While first models aimed only at mathematically describing the passive behaviour of the arterial wall, Holzapfel *et al.* [108], [111] introduced a formulation where model parameters bear a connection to the structural composition of the arterial wall. In particular, the proposed biphasic formulation, discussed in section 3.1 above, accounted for the presence of an isotropic matrix reinforced by two families of fibres oriented symmetrically with respect to the circumferential direction of the vessel conferring the anisotropic and exponential features to the wall tissue. Considering the empirical findings describing collagen-free arterial tissue as almost isotropic [64], [91], the model parameters characterising the isotropic part of the SEF have been related to elastin, while the anisotropic exponential component to collagen fibres [111]. The advantage of this formulation relies on the relatively limited number of unknown parameters (1 parameter for the isotropic part and 4 for the anisotropic part). These parameters need to be fitted on the biaxial experimental data so that the lower the number of parameters, the higher the confidence in their estimation. Some examples of different applications of Holzapfel's model can be found in these works: the regional layer-specific differences in mechanical properties of the porcine aorta [55], nonatherosclerotic thickening of the human coronary wall [112] and human thoracic and abdominal aorta [113]. Figure 5 shows an example of mathematical modelling of the circumferential and axial stress-strain relationship of the swine thoracic aorta using the Holzapfel's strain energy function.

More recently, new SEFs have been introduced to more closely describe the contribution of elastin and collagen to the passive arterial mechanics, including structural proteins volume fractions and stiffness, and collagen fibres orientation and their recruitment through probability functions [100], [101], [109]. While the structural information conveyed by these models is higher compared to simpler models, the number of model parameters necessarily increases while the accuracy of their determination decreases. For example, in the model proposed by Zulliger *et al.* [109], the elastin SEF is $f_{elastin}c_{elastin}(I_1-3)^{\frac{3}{2}}$, where the elastin volume fraction $f_{elastin}$ end the elastin stiffness parameter $c_{elastin}$ are multiplied, leading to an infinite number of combinations of the two parameters providing the same overall result. Therefore, the application of more complex constitutive models and SEFs requires the assumption of some model parameters based on empirical observations (e.g. collagen and elastin volume fractions).

In general, the fitting of the parameters constituting structurally motivated SEFs might provide useful information on the role of elastin and collagen on arterial mechanics. However, the choice of the model necessarily influences the obtained results. For example, Lillie *et al.* [89] fitted a structural based SEF on experimental stress-stretch data of pig aorta and identified a higher stiffness parameter for elastin in the distal portion than in the proximal one, possibly implying different properties of the elastin networks in different regions of the arterial tree. On the contrary, Peña and colleagues [55] explained distal stiffening of the porcine aortic wall with increasing collagen constants using Holzapfel's model, while isotropic constants were unaltered in different regions. Rezakhaniha *et al.* [114] furtherly complicated the modelling of the arterial wall mechanics introducing an anisotropic component for the elastin network and showed a more accurate fitting of the experimental data from the rabbit carotid artery when compared to isotropic elastin SEF proposed by Zulliger, thus highlighting that elastin may have different elastic behaviours in the longitudinal and circumferential direction.

Interestingly, Fonck *et al.* [26] applied SEF to stress-strain relationship obtained from control arteries and arteries subjected

to enzymatic digestion. A change in collagen parameters was observed between elastase treated samples and control ones; more specifically, the strain-recruitment of collagen was altered. In agreement with the works [90], these results suggested a more complex interaction between collagen and elastin compared to the classical view of two constituents acting in parallel.

IV. Conclusion

Structural proteins are present with different concentrations in all three anatomical layers of the arterial wall and the role of each constituent may differ according to its location within the wall thickness. The convoluted structure and the local and regional heterogeneity of arteries make distinguishing the role of collagen and elastin in arterial wall mechanics a complex goal to achieve. Indeed, structural proteins are present in different concentrations in all the three anatomical layers of the arterial wall. Several methods have been applied to achieve this goal, which have been described in this review, together with their results.

Arterial structure, composition and mechanics vary not only at different regions along the arterial tree, but also in different animals [16]. Therefore, comparing results from different species must be done with caution. Additionally, the arterial wall is not a static tissue, is subjected to continuous remodelling with ageing and pathologies in what is thought to be an adaptation process to the changing levels and distribution of stresses throughout the wall thickness [115].

Static microscopy has been used since the 1950s to study arterial mechanics and provided adequate information on the structural alteration of tissue under load. However, the available results this far lack the temporal effect/changes since the sample has to be fixed at a given distending pressure or longitudinal load. Mechanical testing coupled with enzymatic digestion or composition analysis has had limited success as it does not provide direct visualisation of fibres. More recently, advances in microscopy techniques have allowed for fibres imaging and mechanical testing simultaneously, thus providing continuous information on both the response to loading and internal structural changes of a given arterial specimen. Clearly, this last method exploits the major advantages of the earlier proposed techniques and, in the authors' opinion, it provides the best technique to study arterial micromechanics. Furthermore, structural mathematical models based on constitutive equations provide information on the contribution of collagen and elastin to the mechanical behaviour of arteries, however, as with most computational techniques, the choice of the model parameters, which are often assumed, will inevitably influence the obtained results.

The results from all the cited methods have proven the commonly accepted theory that the biphasic shape of the stress-strain relationship of the arterial wall is caused by delayed recruitment of collagen with respect to elastin. Further, the structural reasons behind arterial anisotropicity have been widely investigated. Mechanical testing and enzymatic digestion have shown that collagen confers the anisotropic behaviour to the arterial wall, while elastin has an isotropic response. Also, static and dynamic microscopy have shown a different role of medial and adventitial collagen, with the first playing a role only in the circumferential direction and straightening at high physiological strains, and the latter having a role in both directions and uncrimping at supra-physiological pressures. Indeed, adventitial collagen has a more spread distribution of fibres orientation than medial collagen and seems to be ineffective at physiological pressures, while medial elastin is highly circumferentially oriented. It is of note that some dynamic microscopy results may be biased by the un-physiological loading condition applied in the experiment and, therefore, more work is still necessary to fully understand the role of layer-specific collagen recruitment on arterial anisotropy.

Elastin has been shown to give pseudo-elastic properties to the arterial wall, while collagen network alone showed a highly viscoelastic behaviour. Collectively, elastin and collagen seem to have a complex interconnection, with the first being crucial to give elastic-like properties to the latter. Indeed, several works reported fragmentation of the arterial wall elastin network in case of aneurysms [116]. The loss of the elastin network stability leads to a plastic enlargement of the artery that is consistent with the experimental findings described above.

Overall, advances in microscopy have allowed obtaining reliable methods for the investigation of collagen and elastin micromechanics within the arterial wall; in particular, simultaneous mechanical testing and microscopy imaging is a promising approach. Notwithstanding, to the authors' best knowledge, human arterial samples have been tested to date only in a handful of studies [42], [117]. Clearly, the availability of human donor arteries is limited, but further studies need to be conducted in this direction since inter-mammals differences may lead to wrong interpretations of human arterial mechanics. This may even be even more critical in case a pathological model is studied to draw conclusions on the alteration of arterial micromechanics.

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References

[1] H. Wang *et al.*, "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: A systematic analysis for the Global Burden of Disease Study 2015," *Lancet*, vol. 388, no. 10053, pp. 1459–1544, 2016.

[2] K. D. Kochanek, S. L. Murphy, J. Xu, and B. Tejada-Vera, "Deaths: Final data for 2014," *Natl. Vital Stat. Rep.*, vol. 65, no. 4, pp. 1–122, Jun. 2016.

[3] O. for N. Statistics, "Deaths Registered in England and Wales (Series DR), 2013," 2014.

[4] R. H. Cox, "Passive mechanics and connective tissue composition of canine arteries," *Amer. Physiol. Soc.*, vol. H, pp. 533–541, 1978.

[5] M. L. R. Harkness, R. D. Harkness, and D. A. McDonald, "The collagen and elastin content of the arterial wall in the dog," *Proc. R. Soc. London. Ser. B - Biol. Sci.*, vol. 146, no. 925, pp. 541–551, 1957.

[6] C. M. Kielty, "Elastic fibres in health and disease," *Expert Rev. Mol. Med.*, vol. 8, no. 19, pp. 1–23, 2006.

[7] S. E. Greenwald, "Ageing of the conduit arteries," *J. Pathol.*, vol. 211, no. 2, pp. 157–172, Jan. 2007.

[8] J. C. Kohn, M. C. Lampi, and C. A. Reinhart-King, "Age-related vascular stiffening: Causes and consequences," *Front. Genet.*, vol. 6, 2015, Art. no. 112.

[9] A. Avolio, D. Jones, and M. Tafazzoli-Shadpour, "Quantification of alterations in structure and function of elastin in the arterial media," *Hypertension*, vol. 32, no. 1, pp. 170–175, 1998.

[10] A. C. Burton, "Relation of structure to function of the tissues of the wall of blood vessels," *Physiol. Rev.*, vol. 34, no. 4, pp. 619–642, Oct. 1954.

[11] B. Li and V. Daggett, "Molecular basis for the extensibility of elastin," *J. Muscle Res. Cell Motility*, vol. 23, no. 5–6. pp. 561–573, 2002.

[12] D. J. Prockop and K. I. Kivirikko, "Collagens: Molecular biology, diseases, and potentials for therapy," *Annu. Revis. Biochem.*, vol. 64, pp. 403–434, 1995.

[13] R. L. Armentano *et al.*, "Assessment of elastin and collagen contribution to aortic elasticity in conscious dogs," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 260, no. 6, pp. H1870–H1877, 1991.

[14] P. B. Dobrin, "Mechanical properties of arteries," *Physiol. Rev.*, vol. 58, no. 2, pp. 397–460, 1978.

[15] H. Wolinsky and S. Glagov, "Structural basis for the static mechanical properties of the aortic media," *Circ. Res.*, vol. 14, pp. 400–413, 1964.

[16] H. Wolinsky and S. Glagov, "Comparison of abdominal and thoracic aortic medial structure in mammals," *Circ. Res.*, vol. 25, no. 6, pp. 677–686, Dec. 1969.

[17] B. G. Halloran, V. A. Davis, B. M. McManus, T. G. Lynch, and B. T. Baxter, "Localization of aortic disease is associated with intrinsic differences in aortic structure," *J. Surg. Res.*, vol. 59, pp. 17–22, 1995.

[18] S. Sugita and T. Matsumoto, "Quantitative measurements of the distribution and alignment of collagen fibers in unfixed aortic tissue," *J. Biomech.*, vol. 46, no. 7, pp. 1403–1407, 2013.

[19] M. K. O'Connell *et al.*, "The three-dimensional micro- and nanostructure of the aortic medial lamellar unit measured using 3D confocal and electron microscopy imaging," *Matrix Biol.*, vol. 27, no. 3, pp. 171–181, 2008.

[20] L. H. Timmins, Q. Wu, A. T. Yeh, J. E. J. Moore, and S. E. Greenwald, "Structural inhomogeneity and fiber orientation in the inner arterial media," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 298, no. 5, pp. 1537–1545, 2010.

[21] L. Azinfar, M. Ravanfar, Y. Wang, K. Zhang, D. Duan, and G. Yao, "High resolution imaging of the fibrous microstructure in bovine common carotid artery using optical polarization tractography," *J. Biophotonics*, vol. 10, no. 2, pp. 231–241, Feb. 2017.

[22] H. R. J. Butcher, "The elastic properties of human aortic intima, media and adventitia: The initial effect of thromboendarterectomy," *Ann. Surg.*, vol. 151, pp. 480–489, 1960.

[23] T. Boulesteix *et al.*, "Micrometer scale ex vivo multiphoton imaging of unstained arterial wall structure," *Cytom. Part A*, vol. 69A, no. 1, pp. 20–26, 2006.

[24] K. Wasano and T. Yamamoto, "Tridimensional architecture of elastic tissue in the rat aorta and femoral artery—A scanning electron microscope study," *J. Electron Microsc. (Tokyo).*, vol. 32, no. 1, pp. 33–44, 1983.

[25] M. Raspanti, M. Protasoni, A. Manelli, S. Guizzardi, V. Mantovani, and A. Sala, "The extracellular matrix of the human aortic wall: Ultrastructural observations by FEG-SEM and by tapping-mode AFM," *Micron*, vol. 37, no. 1, pp. 81–86, 2006.

[26] E. Fonck, G. Prod'hom, S. Roy, L. Augsburger, D. A. Rüfenacht, and N. Stergiopulos, "Effect of elastin degradation on carotid wall mechanics as assessed by a constituent-based biomechanical model," *Amer. J. Physiol. - Hear. Circ. Physiol.*, vol. 292, no. 6, pp. 2754–2763, 2007.

[27] J. Clark and S. Glagov, "Transmural organization of the arterial media: The lamellar unit revisited," *Arterioscler. Thromb. Vasc. Biol.*, vol. 5, no. 1, pp. 19–34, Jan. 1985.

[28] T. Shimada, F. Sato, L. Zhang, K. Ina, and H. Kitamura, "Three-dimensional visualization of the aorta and elastic cartilage after removal of extracellular ground substance with a modified NaOH maceration method," *J. Electron Microsc. (Tokyo)*, vol. 42, no. 5, pp. 328–333, Oct. 1993.

[29] K. P. Dingemans, P. Teeling, J. H. Lagendijk, and A. E. Becker, "Extracellular matrix of the human aortic media: An ultrastructural histochemical and immunohistochemical study of the adult aortic media," *Anat. Rec.*, vol. 258, no. 1, pp. 1–14, 2000.

[30] Y. Bezie, P. Lacolley, S. Laurent, and G. Gabella, "Connection of smooth muscle cells to elastic lamellae in aorta of spontaneously hypertensive rats," *Hypertension*, vol. 32, no. 1, pp. 166–169, 1998.

[31] W. Krasny, C. Morin, H. Magoariec, and S. Avril, "A comprehensive study of layer-specific morphological changes in the microstructure of carotid arteries under uniaxial load," *Acta Biomater.*, vol. 57, pp. 342–351, 2017.

[32] S. Polzer *et al.*, "Structure-based constitutive model can accurately predict planar biaxial properties of aortic wall tissue," *Acta Biomater*, vol. 14, pp. 133–145, Mar. 2015.

[33] Y. Zou and Y. Zhang, "An experimental and theoretical study on the anisotropy of elastin network," *Ann. Biomed. Eng.*, vol. 37, no. 8, pp. 1572–1583, Aug. 2009.

[34] R. Rezakhaniha *et al.*, "Experimental investigation of collagen waviness and orientation in the arterial adventitia using confocal laser scanning microscopy," *Biomech. Model. Mechanobiol.*, vol. 11, pp. 461–473, 2012.

[35] P. B. Canham, H. M. Finlay, J. G. Dixon, D. R. Boughner, and A. Chen, "Measurements from light and polarised light microscopy of human coronary arteries fixed at distending pressure," *Cardiovasc. Res.*, vol. 23, no. 11, pp. 973–982, Nov. 1989.

[36] A. J. Schriefl, G. Zeindlinger, D. M. Pierce, P. Regitnig, and G. A. Holzapfel, "Determination of the layer-specific distributed collagen fibre orientations in human thoracic and abdominal aortas and common iliac arteries," *J. R. Soc. Interface*, vol. 9, no. 71, pp. 1275–1286, 2012.

[37] V. Flamini, C. Kerskens, K. M. Moerman, C. K. Simms, and C. Lally, "Imaging arterial fibres using diffusion tensor imaging: Feasibility study and preliminary results," *EURASIP J. Adv. Signal Process.*, vol. 2010, no. 1, pp. 1–13, 2010.

[38] V. Flamini, C. Kerskens, C. Simms, and C. Lally, "Fibre orientation of fresh and frozen porcine aorta determined non-invasively using diffusion tensor imaging," *Med. Eng. Phys.*, vol. 35, no. 6, pp. 765–776, Jun. 2013.

[39] J. T. C. Schrauwen, A. Vilanova, R. Rezakhaniha, N. Stergiopulos, F. N. van de Vosse, and P. H. M. Bovendeerd, "A method for the quantification of the pressure dependent 3D collagen configuration in the arterial adventitia," *J. Struct. Biol.*, vol. 41, no. 7, pp. 1579–1591, 2012.

[40] H. Chen, Y. Liu, M. N. Slipchenko, X. Zhao, J.-X. Cheng, and G. S. Kassab, "The layered structure of coronary adventitia under mechanical load," *Biophys. J.*, vol. 101, no. 11, pp. 2555–2562, 2011.

[41] W. Wan, J. B. Dixon, and R. L. Gleason, "Constitutive modeling of mouse carotid arteries using experimentally measured microstructural parameters," *Biophys. J.*, vol. 102, no. 12, pp. 2916–2925, 2012.

[42] C. Cavinato, C. Helfenstein-Didier, T. Olivier, S. R. du Roscoat, N. Laroche, and P. Badel, "Biaxial loading of arterial tissues with 3D in situ observations of adventitia fibrous microstructure: A method coupling multi-photon confocal microscopy and bulge inflation test," *J. Mech. Behav. Biomed. Mater.*, vol. 74, pp. 488–498, 2017.

[43] H. Chen *et al.*, "Biaxial deformation of collagen and elastin fibers in coronary adventitia," *J. Appl. Physiol.*, vol. 115, no. 11, pp. 1683–1693, 2013.

[44] T. Matsumoto, M. Tsuchida, and M. Sato, "Change in intramural strain distribution in rat aorta due to smooth muscle contraction and relaxation," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 271, no. 4, pp. H1711–H1716, 1996.

[45] N. J. B. Driessen, W. Wilson, C. V. C. Bouten, and F. P. T. Baaijens, "A computational model for collagen fibre remodelling in the arterial wall," *J. Theor. Biol.*, vol. 226, no. 1, pp. 53–64, 2004.

[46] P. Alford, J. Humphrey, and L. Taber, "Growth and remodeling in a thick-walled artery model: Effects of spatial variations in wall constituents," *Biomech. Model. Mechanobiol.*, vol. 7, no. 4, pp. 245–262, Aug. 2008.

[47] A. Tsamis and N. Stergiopulos, "Arterial remodeling in response to hypertension using a constituent based model," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 293, 2007, Art. no. 3130.

[48] J. D. Humphrey, J. F. Eberth, W. W. Dye, and R. L. Gleason, "Fundamental role of axial stress in compensatory adaptations by arteries," *J. Biomech.*, vol. 42, pp. 1–8, 2009.

[49] S. E. Greenwald, J. J. E. Moore, A. Rachev, T. P. C. Kane, and J.-J. Meister, "Experimental investigation of the distribution of the residual strains in the arterial wall," *Trans. ASME*, vol. 119, pp. 438–444, 1997.

[50] R. N. Vaishnav and J. Vossoughi, "Estimation of the residual strains in aortic segments," in *Proc. 2nd Southern Biomed. Eng. Conf., Biomed. Eng. II*, 1983, pp. 330–333.

[51] C. J. Chuong and Y. C. Fung, "On residual stresses in arteries," *J. Biomech. Eng.*, vol. 108, no. 2, pp. 189–192, 1986.

[52] A. Rachev and S. E. Greenwald, "Residual strains in conduit arteries," *J. Biomech.*, vol. 36, no. 5, pp. 661–670, 2003.

[53] V. Alastrué, E. Peña, M. A. Martínez, and M. Doblaré, "Assessing the use of the 'opening angle method' to enforce residual stresses in patient-specific arteries," *Ann. Biomed. Eng.*, vol. 35, no. 10, pp. 1821–1837, Oct. 2007.

[54] J. Humphrey and S. Na, "Elastodynamics and arterial wall stress," *Ann. Biomed. Eng.*, vol. 30, no. 4, pp. 509–523, Apr. 2002.

[55] J. A. Peña, M. A. Martinez, and E. Peña, "Layer-specific residual deformations and uniaxial and biaxial mechanical properties of thoracic porcine aorta," *J. Mech. Behav. Biomed. Mater.*, vol. 50, pp. 55–69, 2015.

[56] N. Stergiopulos, S. Vulliémoz, A. Rachev, J. J. Meister, and S. E. Greenwald, "Assessing the homogeneity of the elastic properties and composition of the pig aortic media," *J. Vasc. Res.*, vol. 38, no. 3, pp. 237–246, May 2001.

[57] G. Holzapfel, G. Sommer, M. Auer, P. Regitnig, and R. Ogden, "Layer-specific 3D residual deformations of human aortas with non-atherosclerotic intimal thickening," *Ann. Biomed. Eng.*, vol. 35, no. 4, pp. 530–545, Apr. 2007.

[58] X. Zheng and J. Ren, "Effects of the three-dimensional residual stresses on the mechanical properties of arterial walls," *J. Theor. Biol.*, vol. 393, pp. 118–126, 2016.

[59] A. Wittek *et al.*, "Cyclic three-dimensional wall motion of the human ascending and abdominal aorta characterized by time-resolved three-dimensional ultrasound speckle tracking," *Biomech. Model. Mechanobiol.*, vol. 15, no. 5, pp. 1375–1388, 2016.

[60] D. H. Bergel, "The dynamic elastic properties of the arterial wall," *J. Physiol.*, vol. 156, no. 3, pp. 458–469, 1961.

[61] D. H. Bergel, "The static elastic properties of the arterial wall," *J. Physiol.*, vol. 156, no. 3, pp. 445–457, 1961.

[62] R. Collins and W. C. L. Hu, "Dynamic deformation experiments on aortic tissue," *J. Biomech.*, vol. 5, no. 4, pp. 333–337, 1972.

[63] Y. C. Fung, K. Fronek, and P. Patitucci, "Pseudoelasticity of arteries and the choice of its mathematical expression," *Amer. J. Physiol.*, vol. 237, no. 5, pp. H620–H631, Nov. 1979.

[64] H. Weisbecker, C. Viertler, D. M. Pierce, and G. A. Holzapfel, "The role of elastin and collagen in the softening behavior of the human thoracic aortic media," *J. Biomech.*, vol. 46, no. 11, pp. 1859–1865, 2013.

[65] D. P. Sokolis, E. M. Kefaloyannis, M. Kouloukoussa, E. Marinos, H. Boudoulas, and P. E. Karayannacos, "A structural basis for the aortic stress–strain relation in uniaxial tension," *J. Biomech.*, vol. 39, no. 9, pp. 1651–1662, 2006.

[66] W. van Gorp, D. S. van I. Schenau, A. P. G. Hoeks, H. A. J. S. Boudier, R. S. Reneman, and J. G. R. De Mey, "Aortic wall properties in normotensive and hypertensive rats of various ages in vivo," *Hypertension*, vol. 26, no. 2, pp. 363–368, 1995.

[67] O. Lichtenstein, M. E. Safar, P. Poitevin, and B. I. Levy, "Biaxial mechanical properties of carotid arteries from normotensive and hypertensive rats," *Hypertension*, vol. 26, no. 1, pp. 15–19, 1995.

[68] J. P. Vande Geest, M. S. Sacks, and D. A. Vorp, "Age dependency of the biaxial biomechanical behavior of human abdominal aorta," *J. Biomech. Eng.*, vol. 126, no. 6, pp. 815–822, Dec. 2004.

[69] M. Shafigh, N. Fatouraee, and A. S. Seddighi, "Determining the biomechanical properties of human intracranial blood vessels through biaxial tensile test and fitting them to a hyperelastic model," *Eng. Solid Mech.*, vol. 1, pp. 43–56, 2013.

[70] D. J. Patel, F. M. De Freitas, J. C. Greenfield, and D. L. Fry, "Relationship of radius to pressure along the aorta in living dogs," *J. Appl. Physiol.*, vol. 18, no. 6, pp. 1111–1117, 1963.

[71] A. Bigi, A. Ripamonti, N. Roveri, G. Jeronimidis, and P. P. Purslow, "Collagen orientation by X-ray pole figures and mechanical properties of media carotid wall," *J. Mater. Sci.*, vol. 16, no. 9, pp. 2557–2562, Sep. 1981.

[72] R. T. Gaul, D. R. Nolan, and C. Lally, "Collagen fibre characterisation in arterial tissue under loading using SALS," *J. Mech. Behav. Biomed. Mater.*, vol. 75, pp. 359–368, 2017.

[73] P. Farand, A. Garon, and G. E. Plante, "Structure of large arteries: Orientation of elastin in rabbit aortic internal elastic lamina and in the elastic lamellae of aortic media," *Microvasc. Res.*, vol. 73, no. 2, pp. 95–99, 2007.

[74] Graham *et al.*, "Localised micro-mechanical stiffening in the ageing aorta," *Mech. Ageing Dev.*, vol. 132, no. 10, pp. 459–467, 2011.

[75] Z. Chang *et al.*, "Nanomechanics and ultrastructure of the internal mammary artery adventitia in patients with low and high pulse wave velocity," *Acta Biomater.*, vol. 73, pp. 437–448, 2018.

[76] R. Akhtar, M. J. Sherratt, R. E. B. Watson, T. Kundu, and B. Derby, "Mapping the micromechanical properties of cryo-sectioned aortic tissue with scanning acoustic microscopy," *Mater. Res. Soc. Symp. Proc.*, vol. 1132, 2009.

[77] R. Akhtar, M. J. Sherratt, J. K. Cruickshank, and B. Derby, "Characterizing the elastic properties of tissues," *Mater. Today*, vol. 14, no. 3, pp. 96–105, 2011.

[78] X. Zhao, R. Akhtar, N. Nijenhuis, and S. J. Wilkinson, "Multi-layer phase analysis: Quantifying the elastic properties of soft tissues and live cells with ultra-high frequency scanning acoustic microscopy," *IEEE Ultrason. Ferroelectr. Freq. Control Soc.*, vol. 59, no. 4, pp. 610–620, Apr. 2012.

[79] R. Akhtar *et al.*, "Frequency-modulated atomic force microscopy localises viscoelastic remodelling in the ageing sheep aorta," *J. Mech. Behav. Biomed. Mater.*, vol. 64, pp. 10–17, Dec. 2016.

[80] J. T. Apter, M. Rabinowitz, and D. H. Cummings, "Correlation of viscoelastic properties of large arteries with microscopic structure," *Circ. Res.*, vol. 19, no. 1, pp. 104–121, 1966.

[81] R. H. Cox, "Comparison of mechanical and chemical properties of extra- and intralobar canine pulmonary arteries," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 242, no. 2, pp. H245–H253, 1982.

[82] R. H. Cox, "Effects of age on the mechanical properties of rat carotid artery," *Amer. J. Physiol.*, vol. 233, no. 2, pp. H256–H263, 1977.

[83] C. L. Berry, S. E. Greenwald, and J. F. Rivett, "Static mechanical properties of the developing and mature rat aorta," *Cardiovasc. Res.*, vol. 9, no. 5, pp. 669–678, 1975.

[84] J. Vassoughi, H. Hedjazi, and F. S. I. Borrism, "Intimal residual stress and strains in large arteries," in *Proc. ASME Bioeng. Conf.*, 1993, pp. 434–437.

[85] S. S. Shahid, R. T. Gaul, C. Kerskens, V. Flamini, and C. Lally, "Quantifying the ultrastructure of carotid artery using high-resolution micro-diffusion tensor imaging: Comparison of intact vs. open cut tissue," *Phys. Med. Biol.*, vol. 62, no. 23, pp. 8850–8868, 2017.

[86] M. R. Roach and A. C. Burton, "The reason for the shape of the distensibility curves of arteries," *Can. J. Biochem. Physiol.*, vol. 35, no. 8, pp. 681–690, Aug. 1957.

[87] P. B. Dobrin and T. R. Canfield, "Elastase, collagenase, and the biaxial elastic properties of dog carotid artery," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 247, no. 1, pp. H124–H131, 1984.

[88] M.-J. Chow, R. Turcotte, C. P. Lin, and Y. Zhang, "Arterial extracellular matrix: A mechanobiological study of the contributions and interactions of elastin and collagen," *Biophys. J.*, vol. 106, no. 12, pp. 2684–2692, 2014.

[89] M. A. Lillie, T. E. Armstrong, S. G. Gérard, R. E. Shadwick, and J. M. Gosline, "Contribution of elastin and collagen to the inflation response of the pig thoracic aorta: Assessing Elastin's role in mechanical homeostasis," *J. Biomech.*, vol. 45, no. 12, pp. 2133–2141, 2012.

[90] S. Roy, T. Thacher, P. Silacci, and N. Stergiopulos, "Arterial biomechanics after destruction of cytoskeleton by Cytochalasin D," *J. Biomech.*, vol. 42, no. 15, pp. 2562–2568, Nov. 2009.

[91] B. Gundiah and Pruitt, "Effects of elastase and collagenase on the nonlinearity and anisotropy of porcine aorta," *Physiol. Meas.*, vol. 34, pp. 1657–1673, 2013.

[92] P. B. Dobrin and W. C. Gley, "Elastase, collagenase and the radial elastic properties of arteries," *Experentia*, vol. 41, pp. 1040–1042, 1985.

[93] A. Harvey, A. C. Montezano, R. A. Lopes, F. Rios, and R. M. Touyz, "Vascular fibrosis in aging and hypertension: Molecular mechanisms and clinical implications," *Can. J. Cardiol.*, vol. 32, no. 5, pp. 659–668, May 2016.

[94] M. Spina, S. Garbisa, J. Hinnie, J. C. Hunter, and A. Serafini-Fracassini, "Age-related changes in composition and mechanical properties of the tunica media of the upper thoracic human aorta," *Arterioscler. Thromb. Vasc. Biol.*, vol. 3, no. 1, pp. 64–76, 1983.

[95] A. Tsamis, J. T. Krawiec, and D. A. Vorp, "Elastin and collagen fibre microstructure of the human aorta in ageing and disease: A review," *J. R. Soc.*, vol. 10, no. 83, pp. 1–22, 2013.

[96] M. Tesauro *et al.*, "Arterial ageing: From endothelial dysfunction to vascular calcification," *J. Intern. Med.*, vol. 281, no. 5, pp. 471–482, May 2017.

[97] D. Haskett, G. Johnson, A. Zhou, U. Utzinger, and J. Vande Geest, "Microstructural and biomechanical alterations of the human aorta as a function of age and location," *Biomech. Model. Mechanobiol.*, vol. 9, no. 6, pp. 725–736, Dec. 2010.

[98] H. Bader, "Dependence of wall stress in the human thoracic aorta on age and pressure," *Circ. Res.*, vol. 20, pp. 354–361, 1967.

[99] T. C. Gasser, R. W. Ogden, and G. A. Holzapfel, "Hyperelastic modelling of arterial layers with distributed collagen fibre orientations," *J. R. Soc.*, vol. 3, pp. 15–35, 2006.

[100] M. A. Zulliger and N. Stergiopulos, "Structural strain energy function applied to the ageing of the human aorta," *J. Biomech.*, vol. 40, no. 14, pp. 3061–3069, 2007.

[101] Y. Wang, S. Zeinali-Davarani, and Y. Zhang, "Arterial mechanics considering the structural and mechanical contributions of ECM constituents," *J. Biomech.*, vol. 49, no. 12, pp. 2358–2365, 2016.

[102] J. A. Kratzberg, P. J. Walker, E. Rikkers, and M. L. Raghavan, "The effect of proteolytic treatment on plastic deformation of porcine aortic tissue," *J. Mech. Behav. Biomed. Mater.*, vol. 2, no. 1, pp. 65–72, 2009.

[103] R. H. Cox, "Comparison of arterial wall mechanics using ring and cylindrical segments," *Amer. J. Physiol. Hear. Circ. Physiol.*, vol. 244, no. 2, pp. H298–H303, 1983.
[104] A. Zoumi, X. Lu, G. S. Kassab, and B. J. Tromberg, "Imaging coronary artery microstructure using second-harmonic and two-photon fluorescence microscopy," *Biophys. J.*, vol. 87, no. 4, pp. 2778–2786, 2004.
[105] S. Sugita and T. Matsumoto, "Multiphoton microscopy observations of 3D elastin and collagen fiber microstructure changes during pressurization in aortic media," *Biomech. Model. Mechanobiol.*, vol. 16, no. 3, pp. 763–773, 2016.
[106] W. Krasny, H. Magoariec, C. Morin, and S. Avril, "Kinematics of collagen fibers in carotid arteries under tension-inflation loading," *J. Mech. Behav. Biomed. Mater.*, vol. 77, pp. 718–726, 2018.
[107] A. J. Schriefl, H. Wolinski, P. Regitnig, S. D. Kohlwein, and G. A. Holzapfel, "An automated approach for three-dimensional quantification of fibrillar structures in optically cleared soft biological tissues," *J. R. Soc. Interface*, vol. 10, no. 80, 2013, Art. no. 20120760.
[108] G. Holzapfel, T. Gasser, and R. Ogden, "A new constitutive framework for arterial wall mechanics and a comparative study of material models," *J. Elast.*, vol. 61, no. 1, pp. 1–48, Jul. 2000.
[109] M. A. Zulliger, P. Fridez, K. Hayashi, and N. Stergiopulos, "A strain energy function for arteries accounting for wall composition and structure," *J. Biomech.*, vol. 37, no. 7, pp. 989–1000, Jul. 2004.
[110] R. N. Vaishnav, J. T. Young, and D. J. Patel, "Distribution of stresses and of strain-energy density through the wall thickness in a canine aortic segment," *Circ. Res.*, vol. 32, pp. 577–583, 1973.
[111] G. A. Holzapfel and H. W. Weizsäcker, "Biomechanical behavior of the arterial wall and its numerical characterization," *Comput. Biol. Med.*, vol. 28, no. 4, pp. 377–392, 1998.
[112] G. A. Holzapfel, C. T. Gasser, G. Sommer, and P. Regitnig, "Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling," *Amer. J. Physiol.*, vol. 289, no. 5, 2005, Article no. H2048.
[113] H. Weisbecker, D. M. Pierce, P. Regitnig, and G. A. Holzapfel, "Layer-specific damage experiments and modeling of human thoracic and abdominal aortas with non-atherosclerotic intimal thickening," *J. Mech. Behav. Biomed. Mater.*, vol. 12, pp. 93–106, 2012.
[114] R. Rezakhaniha, E. Fonck, C. Genoud, and N. Stergiopulos, "Role of elastin anisotropy in structural strain energy functions of arterial tissue," *Biomech. Model. Mechanobiol.*, vol. 10, no. 4, pp. 599–611, Jul. 2011.
[115] A. Saini, C. Berry, and S. Greenwald, "The effect of age and sex on residual stress," *J. Vasc. Res.*, vol. 32, pp. 398–405, 1995.
[116] B. T. Baxter, V. A. Davis, D. J. Minion, Y. P. Wang, T. G. Lynch, and B. M. McManus, "Abdominal aortic aneurysms are associated with altered matrix proteins of the nonaneurysmal aortic segments," *J. Vasc. Surg.*, vol. 19, no. 5, pp. 797–803, 1994.
[117] J. S. Bell *et al.*, "Microstructure and mechanics of human resistance arteries," *Amer. J. Physiol. Heart. Circ. Physiol.*, vol. 311, no. 6, pp. H1560–H1568, 2016.

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