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Biochemomechanics of the thoracic aorta in health and disease

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Abstract

Aneurysms and dissections of the thoracic aorta are life threatening events with poorly understood pathophysiologies which may have genetic origins. By starting with an introduction to these pathologies, we focus on the biochemomechanics of the healthy thoracic aorta. Specifically, we describe the microstructure and the mechanics of the aortic tissue since it is known that the microstructure strongly influences the biomechanical behavior. This relationship is then complemented by providing more detailed information on the selected extracellular matrix components (collagen, elastic fibers and proteoglycans) and smooth muscle cells. More specifically, we introduce the roles smooth muscle cells play in the function of the aortic wall: actively (mechanically) with their contractile abilities and passively by regulating the composition of the extracellular matrix they are embedded in, in particular via the transforming growth factor β (TGF-β) pathway. Subsequently, we summarize the microstructural changes in thoracic aortic aneurysms and dissections in connection with selected risk factors and genetic mutations, and couple these changes with the findings on the biomechanical behavior of the pathological tissues. Finally, we provide a summary and concluding remarks.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide and is expected to account for more than 23 million deaths by 2030 [1]. Two of these diseases that occur in the thoracic aorta (TA)—aneurysms (TAA) and dissections (TAD)—are rare but life threatening events with poorly understood pathophysiologies. Mortality rates approach 50% within the first 48 hours and 80% within 2 weeks for the dissections of the ascending aorta that are left untreated [2], and the perioperative mortality rates for intact and ruptured TAA are 6.1% and 28%, respectively [1].

Aneurysms are defined as dilatations of arteries when the diameter of the affected segment exceeds 1.5 times the normal diameter [3]. According to the morphology, aneurysms can be either saccular or fusiform, the latter being more common in the aorta. They can also be classified according to the aortic segment involvement as thoracic, thoracoabdominal or abdominal. They are typically silent until they dissect or rupture.

Acute aortic dissections, unlike aneurysms, are not silent (85%) and most patients report severe pain [3]. According to the Stanford classification, an aortic dissection can be either of type A—involving the ascending aorta—or of type B—affecting only the descending TA and below. The term non-A non-B aortic dissection was coined for the descending aortic dissections extending into the aortic arch or the ascending aorta [4]. Type B dissection is generally treated pharmacologically, whereas type A dissection is treated surgically. Dissections of the aorta typically involve a tear in the intima where blood can flow into and initiate an intimal flap. Separation of the wall proceeds, either antegrade or retrograde, within the media creating a false lumen. The false lumen can lead to a dilatation of the aorta, and in some cases block the true lumen (true-lumen collapse, see, for example, [5]) impairing the blood flow significantly. Although it is typical, the aforementioned intimal flap is not always present. Dissections can also be caused by intramural hematoma which is located around the circumference of the aorta or by penetrating aortic ulcers [3], as depicted in figure 1.

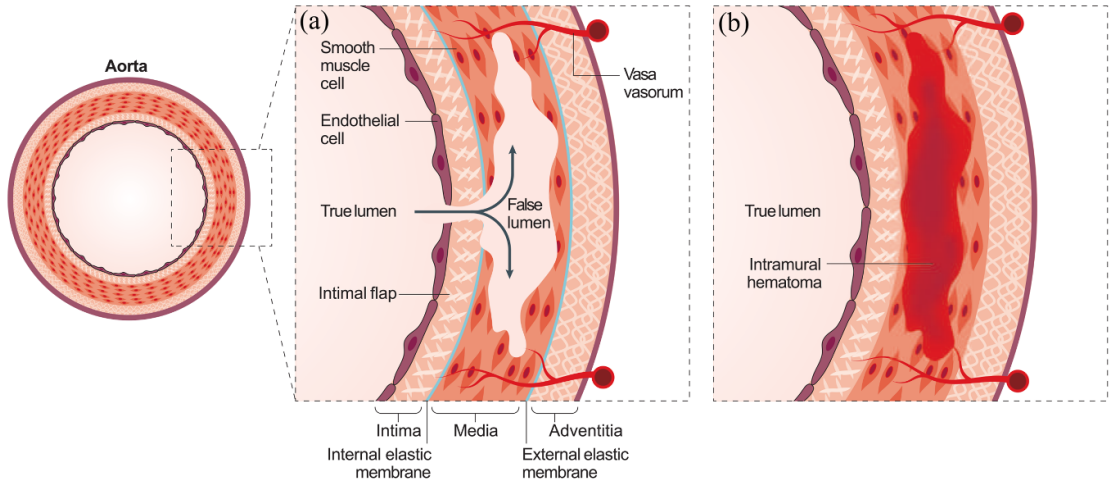

Figure 1. Aortic dissection: (a) resulting from an intimal tear (most cases) and creating an intimal flap and a false lumen; (b) resulting from a rupture of the vasa vasorum (some cases) and leading to an intramural hematoma that may progress to form an aortic dissection; [7] copyright © 2016, Springer Nature.

It is important to note here, that although aneurysms can dissect, they are not a prerequisite for dissection to occur. In fact, more than 80% of all dissections occur in the absence of a pre-existing aneurysm [6]. Except where a distinction is made, thoracic aortic aneurysms and dissections are referred to as TAAD from hereon.

The major risk factor is hypertension for aneurysms and dissections of the ascending thoracic aorta similar to other CVDs, whereas the second major risk factor is inheritance [3, 8, 9]. Abdominal and descending thoracic aortic aneurysms, on the other hand, have more overlapping risk factors with CVDs and are commonly accompanied by atherosclerosis [3, 10]. The pathophysiology of aneurysms differ not only between the abdominal aorta (AA) and the thoracic aorta (TA) [11, 12], but also between descending and ascending portions of the thoracic aorta [13]. Such differences are typically attributed to the embryonic origins of the cells involved in the process as there might be differences in how the signaling pathways function [11, 13]. This can provide an explanation for why aneurysms in the ascending aorta grow somehow slower than the aneurysms of the descending TA [3].

TAAD can be divided into syndromic and nonsyndromic types, while the latter can be either familial or sporadic. The nonsyndromic familial type is the one in which more than one family member is effected, whereas in the nonsyndromic sporadic type no other family member is affected [14]. Genetic mutations are typically the cause of both syndromic and nonsyndromic familial types [15] and mutations in 13 genes have been shown to predispose to thoracic aortic disease so far [16]. More specifically, syndromic TAADs are usually caused by mutations to the genes which are widely expressed in the body, whereas the nonsyndromic familial ones result from mutations to the proteins with specific functions in the aorta, especially to the smooth muscle cell (SMC) specific proteins [17].

Parts of the TA affected by aneurysms and/or dissections are typically treated surgically when certain criteria are met. The decision is mainly based on the critical diameter among others such as yearly growth rate. The patient suffering from an asymptomatic aneurysm is operated on to prevent it from rupturing or dissecting if the diameter is 5 and 5.5 cm for men and women, respectively [19]. These diameter values are recommended since the statistical analyses show hinge points where dissection and rupture become common events rather than rarities, at a diameter of 6 cm for the ascending and 7 cm for the descending TA [20], see also figure 2(a)–(b). If the patient suffers from a TAD or a symptomatic TAA the affected portion is suggested to be resected regardless of the size [21]. In case the patient has a connective tissue disorder such as Marfan syndrome or Ehlers-Danlos syndrome due to a mutation, the risk of suffering from aneurysms or dissections can increase substantially as well as the risk of adverse events. Hence, the recommendations for surgical intervention also reflect the observations from such mutations, see figure 2(c). Covering all genetic mutations related to TAADs would be out of the scope of this review article, however, some of the selected mutations are briefly described in section 3.1 and an extended list of mutations related to TAAD can be found in [9, 10, 17, 18].

The following sections aim to present the relevant information on the structure and mechanical behavior of the thoracic aorta in health and disease. More specifically, section 2 provides an overview of the healthy aortic wall microstructure and its mechanical behavior, presents detailed information on extracellular matrix (ECM) components, how SMCs are involved in the aortic wall function and how they take part in regulating

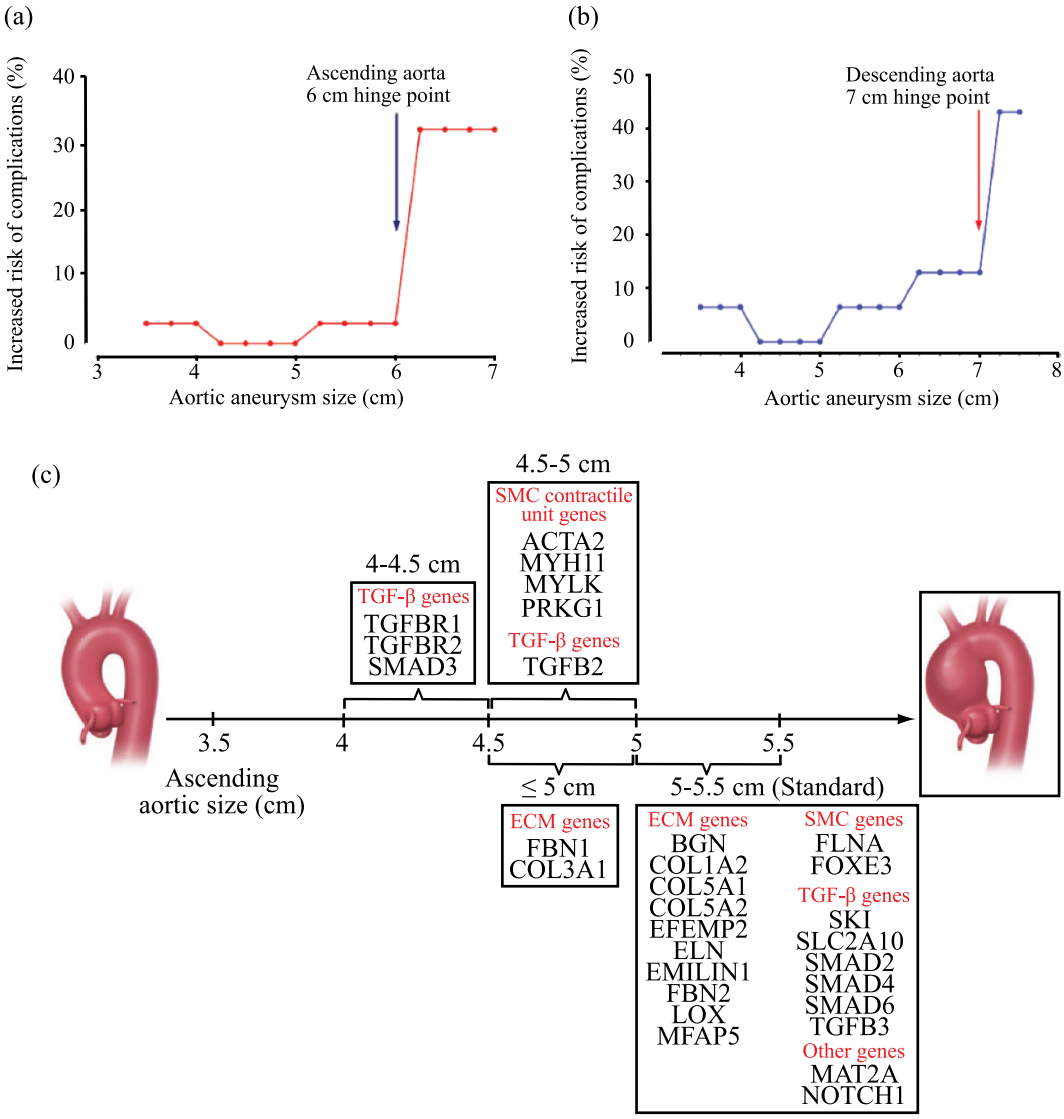

Figure 2. Increased risk of complications (in %) in relation to aneurysm diameter (in cm) in (a) the ascending and (b) the descending thoracic aorta; copyright © 2017, Springer Nature [13]. (c) Recommended size for surgical intervention on the ascending thoracic aorta depend on the mutations the patient may suffer from; [18] copyright © 2018 by Thieme Medical Publishers, Inc.

the ECM they are embedded in. Section 3 begins with a summary of pathological structural changes and selected non-genetic and genetic risk factors that attracted attention over the past years and have common structural manifestations. Subsequently, pathological structural changes in the TAADs are connected to the biomechanical characteristics of the diseased thoracic aortic tissues. In section 4, we conclude with a summary and future research perspectives.

2. Healthy aortic wall

Intramural cells—endothelial cells, SMCs, fibroblasts—and the ECM produced by these cells give rise to the aorta's layer-specific mechanical properties and functions. To keep functional homeostasis, it is vital for the cells to sense their environment by interacting with each other and the ECM components to control the ECM organization: SMCs can contract or relax in response to changes in their biochemomechanical environment; and all intramural cells play a role in the synthesis and degradation of the ECM components. For example, an increase in the local forces applied by the ECM proteins at the cell junctions can activate signal transduction pathways, upon which a cascade of anabolic (matrix building) or catabolic (matrix degrading) reactions are triggered.

This section begins with the microstructure and the mechanical behavior of aortic walls. Subsequently, detailed information on the structural components of the wall that drew attention in relation to the pathological aortic wall—collagen, elastic fibers and proteoglycans—is provided. Next, mechanisms of SMC

contraction and the regulation of the ECM through mechanotransduction are introduced, since sustained disruptions to the preferred mechanical environment of cells may lead to pathological remodeling of the ECM, and the components of the ECM have a key influence on the mechanical behavior of the tissue.

2.1. Microstructure

The aortic wall constitutes of three layers: intima—the innermost layer, media, and adventitia—the outermost layer. The media is separated from the intima and adventitia via internal and external elastic lamina, respectively.

The *intima* of a young healthy adult reaches until the internal elastic lamina and consists of a single layer of endothelial cells, a thin basal lamina and a subendothelial area. Basal lamina is a sheet-like structure formed by laminins, type IV collagen, perlecan and other gylcoproteins binding together [22]. The subendothelium develops gradually with age and it is supported by the internal elastic lamina [23]. In large elastic arteries of humans, the fibrous subendothelium may also contain vascular smooth muscle cells [23, 24]. Mechanical contribution of the intima as a load bearing component is negligible in a young healthy adult. However, this layer becomes mechanically significant with age due to non-atherosclerotic intimal thickening [24, 25].

The human aortic *media* is composed of 28–60 concentric medial lamellar units (MLUs) attached together. During the prenatal period, growth of the AA and the TA is similar: the number of MLU increases until a relatively constant tension per MLU is reached [26], while maintaining the ratio of aortic diameter to medial thickness [27]. During the postnatal period, the ratio of aortic diameter to medial thickness is preserved as the blood pressure, tension per MLU and the segment diameters increase [27]. However, medial growth in the TA is achieved by an increase in the number of MLU, whereas in the AA it is mainly achieved by an increase in the MLU thickness. During this growth, tension per MLU becomes greater in the AA than in the TA maintaining a relatively similar wall stress per MLU along the aorta [27]. The analysis documented in [28] showed that the number of MLU in both segments increases exponentially with increasing stroke volume, and the number of MLU in the AA is linearly related to the pulse pressure. The recent study [29] reported increased aortic wall thickness and radii with increasing age accompanied by a decrease in medial elastin, while the number of MLU and physiological circumferential stress per MLU remained constant.

A single MLU consists of a fenestrated elastic sheet upon which radially tilted circumferentially oriented SMCs are lying embedded in a network of collagen and elastin [30]. Orientations of SMC nuclei and collagen fibers in a single unit correlate and are closer to the circumferential direction [31]. When the entire thickness of the medial layer is considered, collagen fibers exhibit helically arranged two symmetric families which are dispersed around a mean orientation closer to the circumferential direction [32]. Predominantly, type III (70%) and type I (30%) fibrillar collagens are found in this layer, but fibril forming collagen type V and network forming collagen type IV are also present [33, 34].

The two symmetric collagen fiber families in the *adventitia* are dispersed around a mean fiber angle closer to the longitudinal direction, which is in contrast with the media [32]. In addition to thick collagen fiber bundles that are rich in type I collagen [33], fibroblasts and some elastic fibers are found in this layer.

2.2. Mechanical behavior

The typical mechanical behavior of the aortic wall with respect to stress-stretch is highly *nonlinear*. In other words, the tissue stiffens progressively with increasing applied load. The nearly linear stress-stretch behavior at a lower load level is governed by the elastin, whereas collagen governs the non-linear tissue behavior where the artery exhibits a typical J-shaped response curve at higher loads [35]. The blue curve in figure 3(a) depicts such a typical behavior for an aortic tissue strip stretched in one direction, and the black curve therein depicts the almost linear response of the tissue upon collagenase treatment [36]. Gradual employment—due to uncrimping and reorientation towards the loading direction—of collagen fibers embedded in the tissue with increasing load is responsible for the non-linearity, whereas the preferred direction of the fibers results in *anisotropy* [37]. Human descending thoracic aortas become stiffer, more nonlinear and anisotropic with advanced age [29].

The study [38] revealed that glycosaminoglycans (GAGs) play a role in collagen recruitment. Porcine descending aortas enzymatically treated for GAG removal exhibited earlier stiffening under biaxial loading, as well as reduced percent stress relaxation and rate of relaxation in both circumferential and longitudinal directions compared with untreated tissues. GAG removal resulted in minimal changes to fiber reorientation under non-equibiaxial deformation compared with non-treated tissues, however, there were differences in collagen fiber recruitment and waviness. More specifically, all collagen fibers were recruited earlier and collagen fibers in the adventitia were significantly more straight in the treated tissues compared with [39] which reported early elastin, continuous medial collagen and delayed adventitial collagen recruitment for untreated tissues.

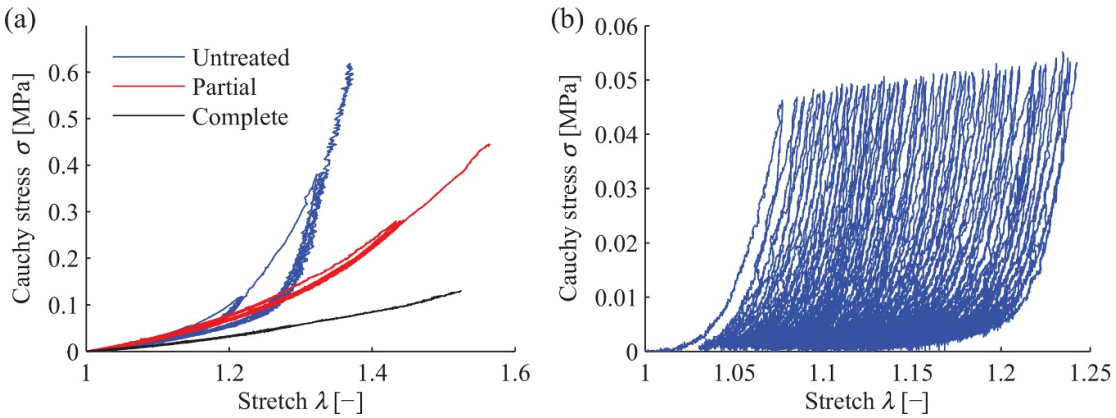

Figure 3. Stress-stretch behavior of aortic tissue strips: (a) mechanical behavior becomes gradually linear, and the hysteresis decreases upon treatment with collagenase; (b) tissue keeps getting softer with each loading cycle upon elastase treatment during preconditioning; reprinted from [36], copyright (2013), with permission from Elsevier.

Furthermore, due to its high water content hold by the proteoglycans (PGs), aortic tissues can be assumed to undergo volume preserving deformation in response to mechanical load: it is *incompressible* [40–42]. However, incompressibility assumption may not be valid at the micromechanical level [43, 44].

The aortic wall is also *pseudoelastic* – it follows different paths during loading and subsequent unloading. The difference in the loading-unloading paths is called hysteresis, and it can be reduced by applying several loading-unloading cycles after which mechanical response becomes repetitive; upon which the tissue is said to be preconditioned [45]. Strips of human thoracic aortas with non-atherosclerotic intimal thickening treated with elastase to degrade elastin exhibited an increased softening with each additional load cycle [36], see figure 3(b). In other words, the behavior did not become repetitive after applying consecutive loading-unloading cycles, indicating that the integrity of the aortic wall was reduced upon elastase treatment. This observation was attributed to the collagen fiber network being inadequately connected to the non-collagenous matrix in the elastase treated tissue, in which collagen fibers were able to slide against each other [36].

In addition, when an unloaded arterial ring is cut radially it springs open and a longitudinal strip bends further away from the main vessel axis. This implies that the external part of the artery is under tension while the internal part is under compression, see [46] and references therein. In other words, arteries are *residually stressed* in both circumferential and longitudinal directions *in vivo*, for which elastin is mainly responsible [47–49]. Residual stresses are layer dependent [50]; they are more concentrated at the inner parts of the arteries where more elastin is found. In the outer layers, however, they contribute little to the stress state [50]. They are reported to increase as one moves away from the heart [49], however, in [51] the highest residual stresses are documented at the aortic arch, declining sharply toward the ascending aorta while declining to an almost constant value in the descending aorta, and increasing again in the abdominal aorta. Furthermore, residual stresses increase with increasing age [49, 51]. It was suggested that these stresses are generated as a result of non-uniform growth [52, 53], they are necessary for 'compatible growth' [54], and they homogenize the stress gradients across the wall [55]. The study [56] hypothesized that the transmural distribution of charged PGs plays a significant role in regulating residual stresses in arterial walls. This hypothesis was tested theoretically and verified experimentally by measuring opening angles of rat aortas in NaCl solutions of various ionic strengths. This study suggests that arterial walls remodel in response to changes in wall stresses by altered deposition of PGs across the wall thickness.

By maintaining a basal tone and thereby introducing residual strains (hence stresses), SMCs can also attenuate the stress gradients as well as the stress levels [57, 58]. For example, an increase in the basal tone causes an increase in the arterial pressure at which circumferential strain distribution throughout the wall thickness can be kept uniform under physiological conditions [57–60]. It is worth noting that the influence of SMC tone on the residual stresses in the human aorta needs to be further investigated, since the study [57] was conducted on rat thoracic aortas. Regardless, SMCs control the lumen diameter regulating the circumferential and shear stresses acting on the wall via their active response, and produce the ECM they are embedded in.

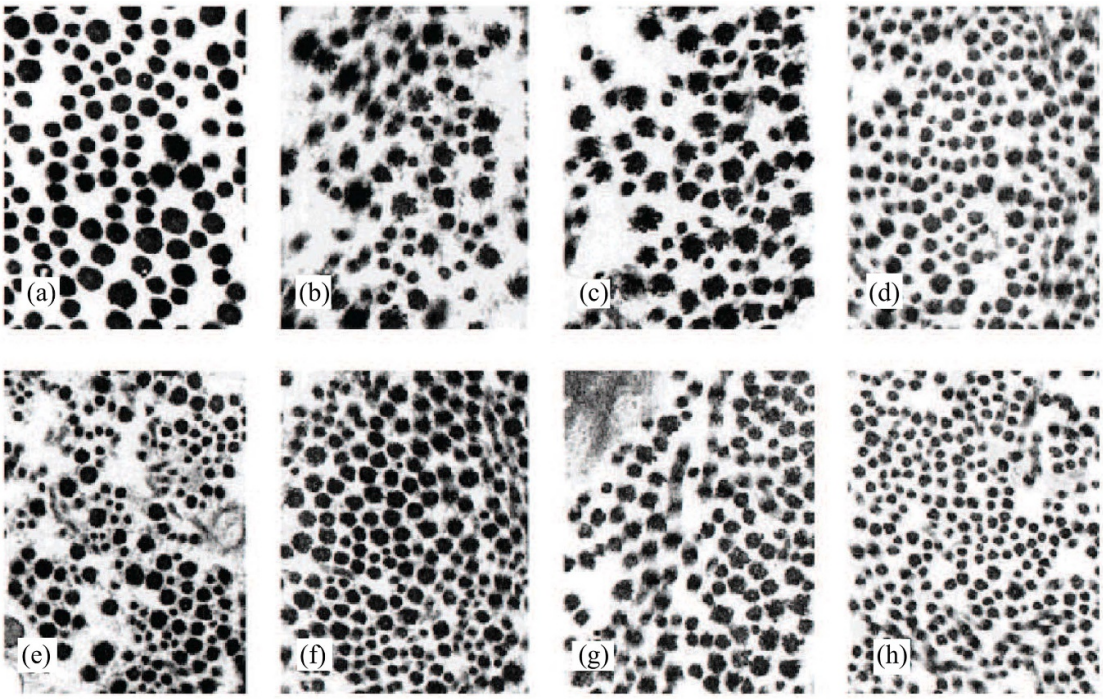

Figure 4. Fibril diameters in the aortic wall: (a) adventitia with thick fibrils; (b)–(g) media with variable fibril diameters, with the adventitial side containing fibrils with significantly larger diameter compared to the intimal side; (h) intima with thin fibrils (all images × 35 000); [34] John Wiley & Sons. Copyright © 2000 Wiley-Liss, Inc.

2.3. ECM components

As introduced in section 2.2, the components of the ECM, their orientations and interplay determine the mechanical behavior of the tissue. In this section, we take a closer look into some of these ECM components such as collagen, elastic fibers and proteoglycans. In particular, we describe the hierarchical structure formation for collagen and elastic fibers, and continue with the role of proteoglycans in that formation.

2.3.1. Collagen

Collagen, with 28 types identified in humans so far, is the most abundant protein in the body [61]. In addition to strengthening the tissue, collagen sequesters cytokines and mediates cellular activities via its connections to cell surface receptors [62]. Fibrillar collagens form highly hierarchical structures. Bundles of fibers such as in tendons can have diameters up to 500 μm, a single fiber that consists of several fibrils can have diameters in the range of 1-20 μm, and a single fibril can have diameters in the range of 10-300 nm [63].

In the human aorta, collagen fibril diameters are reported to be smallest in the intima (37.36 nm) and largest in the adventitia (46.58 nm), whereas within the media they are heterogeneous, even within a fiber [34], see figure 4. In addition to the preferred direction of fibers, which provide strength and resilience along the main direction of the *in vivo* load, fibrils with different diameters that constitute a fiber enable the fiber to exhibit high tensile strength and high creep resistance. Larger diameter fibrils in the tissue serve to increase the tensile strength of a fiber with their larger cross-sectional areas, as it is the case for the adventitia, whereas the smaller diameter fibrils ensure an increased inter-fibril binding due to their high surface to volume ratio [64].

Fibrils are formed via axially shifted lateral stacking of tropocollagen helices, as depicted in figure 5(a). Owing to the axial shift in the regular stacking, they exhibit a periodic structure with a d-period of approximately 67 nm [65, 66], see figure 5(a)–(b). The d-period consists of a gap and overlap zone of 35 and 32 nm, respectively [67], and its contribution to tissue level stretch is approximately one order of magnitude lower than the macroscopic stretch [68].

Each tropocollagen molecule is composed of three α chains each of which contain a distinct amino acid triplet repeat, Gly-X-Y. Upon being synthesized, α chains are imported into the rough endoplasmic reticulum [63], where they undergo hydroxylation that forms hydrogen bonds between the chains. The end-product is a triple helix called tropocollagen with N- and C- terminal domains [66], see figure 5(c). Depending on the distinguishable chain types present in a collagen molecule, it can be classified as a homotrimer with three identical chains, or heterotrimer with two or three distinct chains [70].

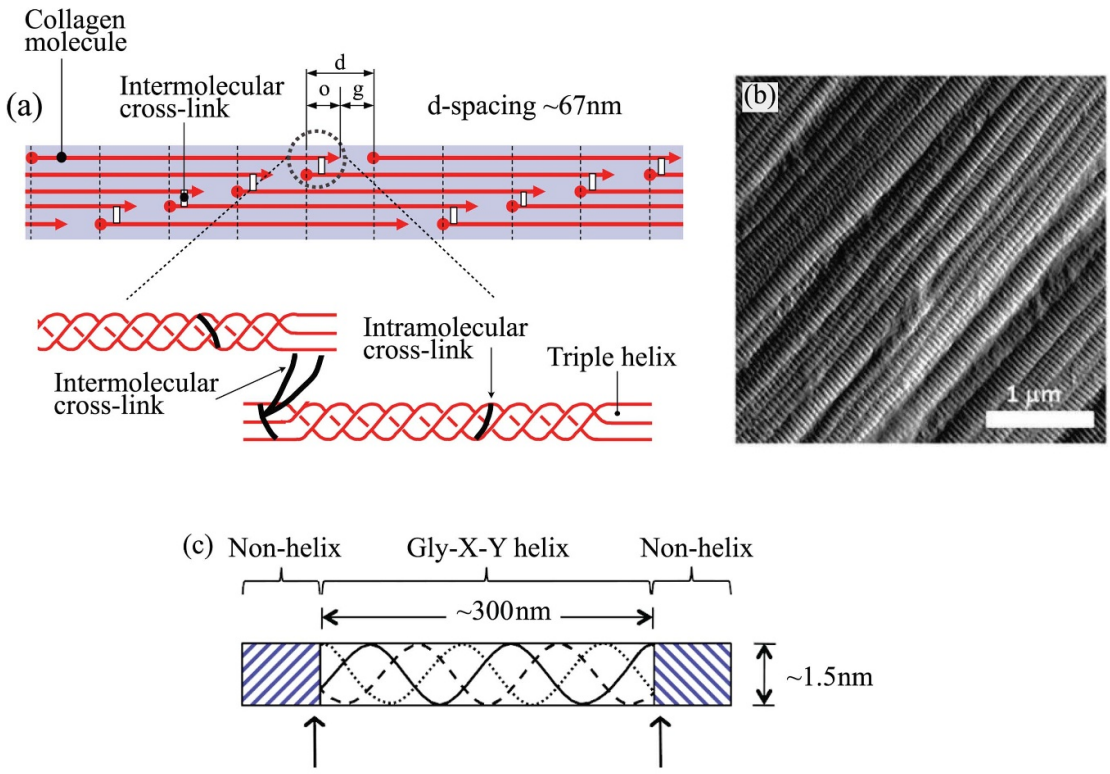

Figure 5. From tropocollagen to fibril: (a) schematic arrangement of collagen molecules linked to each other via covalent cross-links in a fibril unit cell exhibiting a d-spacing (staggering period) of about 67 nm, with an overlap of the molecules labeled o, and a gap region labeled g; (b) electron micrograph of a tendon clearly showing the periodic structure of collagen fibrils (reproduced from [69]. CC BY 3.0); (c) schematic of a fibril forming procollagen molecule with shaded areas depicting non-helical N- and C- terminal ends, arrows indicate enzymatic cleavage sites; reprinted from [63], copyright (2013), with permission from Elsevier.

While procollagen molecules are being transported from the endoplasmic reticulum by Golgi-to-plasma membrane carriers to the ECM, procollagens are converted to tropocollagens following enzymatic removal of C-domains by procollagen C-proteinases exposing C-telopeptides, and fibrils can already start forming [71]. Golgi to plasma membrane carrier pushes out the cell membrane creating a 'fibripositor' (fibril depositor), which fuses into the membrane, creates an opening and subsequently deposits the fibril into the ECM [71, 72]. Stable assembly of fibrils require lysyl oxidase to cross-link lysine and hydroxylysine residues at the N- and C-telopeptides covalently [63].

Type I collagen can form fibrils by self-assembly *in vitro*, however, proper *in vivo* assembly requires fibronectin, integrins that bind to fibronectin and collagen, as well as collagen types III and V [73]. In the absence of types III [74] and V [75] collagen, type I rich fibrils have larger and inconsistent diameters. In the arteries, collagen types III and V are co-localized with collagen type I near the elastic lamina [34].

Forming inter-fibril cross-links and cross-links with other matrix constituents contribute to a stable and functional matrix formation. Fibrils are cross-linked to each other on the fibril surface by small leucine rich proteoglycans to form fibers [76]. Different types of leucine rich proteoglycans are involved in different stages of fibril formation depending on the fibril requirements. Their binding to collagen molecules could (i) prevent uncontrolled fibril/fiber assembly by sterical hindrance, (ii) bridge different types of collagen, (iii) regulate cross-linking on each collagen monomer, or all [77].

2.3.2. Elastic fibers

Functional elastic fibers contribute to the compliance and resilience of the aortic wall, and damage to them results in irreversible changes in function and wall structure [78]. They consist of cross-linked elastin (~90%) covered by fibrillin microfibrils (~10%). Figure 6(a) depicts the multiscale structure of elastic fibers together with elastic fiber-associated proteins such as microfibril-associated protein, elastin-microfibril interface-located proteins and microfibril-associated glycoprotein.

Elastin is the main protein of elastic fibers, and it is secreted as tropoelastin by fibroblasts and SMCs upon gene activation in response to stimulants such as insulin-like growth factor and nitric oxide (NO). Tropoelastin monomers bind to elastin-binding protein (EBP) prior to being secreted into the ECM, which protects tropoelastin from proteolysis. Furthermore, EBP aids the cross-linking by lysyl oxidase and assembly

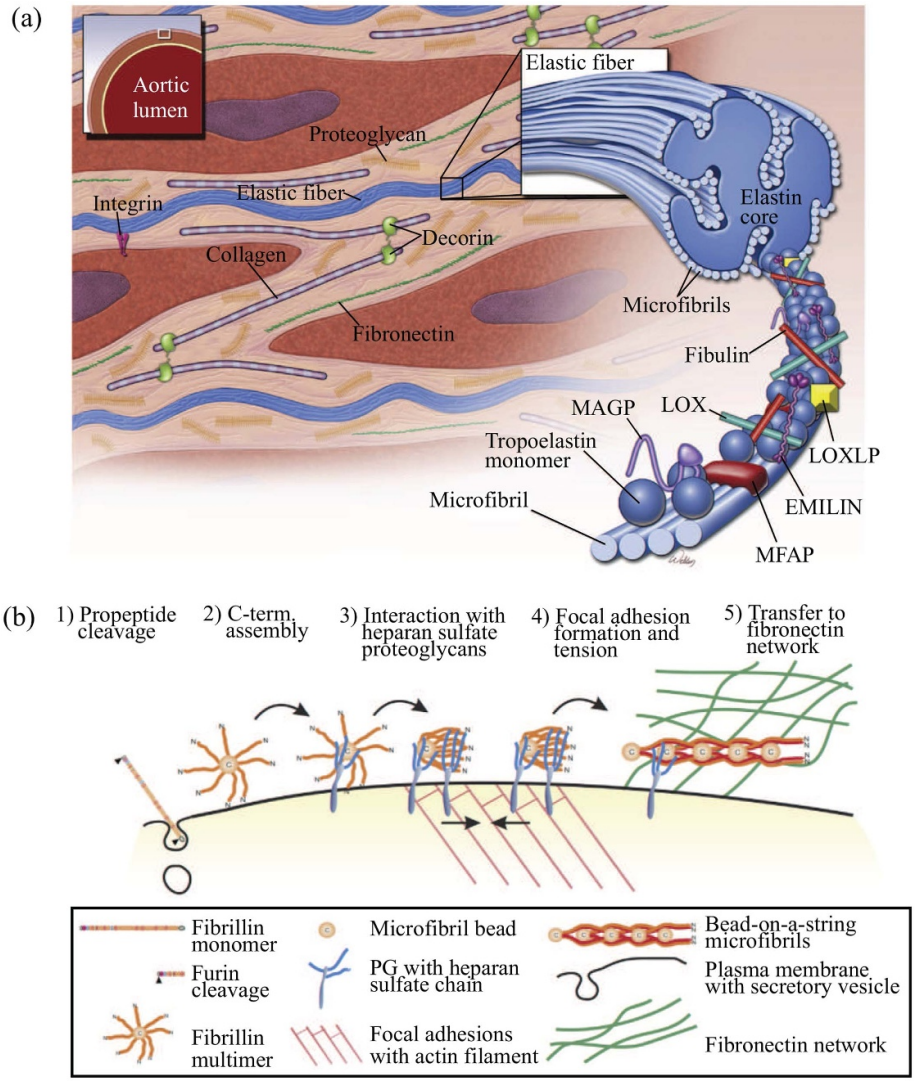

Figure 6. Elastic fiber structure in the medial layer of the aorta: (a) elastin core consists of tropoelastin monomers connected by fibulins and cross-linked by lysyl oxidase (LOX) and lysyl oxidase-like protein (LOXLP). Microfibrils contain fibrillins, microfibril-associated glycoprotein (MAGP) and microfibril-associated protein (MFAP). The core is attached to microfibrils by elastin microfibril interface-located proteins (EMILINs) forming an elastic fiber; reprinted from [62], Copyright 2013), with permission from Elsevier. Model of microfibril assembly: (b) fibrillin monomers are secreted to the ECM where they first self assemble, and then assemble into bead on a string structure with the help of heparan sulfate proteoglycans and fibronectin; [79] John Wiley & Sons. © 2015 Federation of European Biochemical Societies.

of elastin onto the microfibrils. Upon the detachment of EBP from tropoelastin, a self-organized globular aggregate forms on the cell surface—a process called microassembly. Fibulins (fibulin-4 and fibulin-5) bind to this aggregate and mediate its deposition onto microfibrils. During the macroassembly, the mature insoluble elastic fiber is formed via enzymatic cross-linking of lysyl oxidase and lysyl oxidase-like protein [63, 80, 81]. For a recent review on the theories regarding the assembly of elastic fibers the reader is referred to [82].

Fibrillin rich microfibrils have a repeated beaded structure with a periodicity of 56 nm [83], and they consist of mainly fibrillins and microfibril-associated glycoproteins. Fibrillins (fibrillin-1, -2, -3) are large glycoproteins which become rod-like in the presence of Ca^{2+} [84]. Figure 6(b) depicts a model of microfibril formation. Upon the secretion from the cell, fibrillin monomers self-assemble into multimers. Following the interaction with heparan sulfate proteoglycans at focal adhesions, they can be formed into bead-like multimers. Connection with fibronectin fibers help microfibrils stabilize, elongate and interact with other ECM proteins [79].

Although microfibrils surround the elastin core and provide a scaffold for tropoelastin deposition and organization, their role is not limited to elastic fiber formation [81]. They can also form connections devoid of elastin (not vice versa), for example, between SMCs and elastic lamellae, and between the lamellae [85]. In addition, microfibrils interact with other elastic fiber associated proteins and molecules such as latent TGF-β

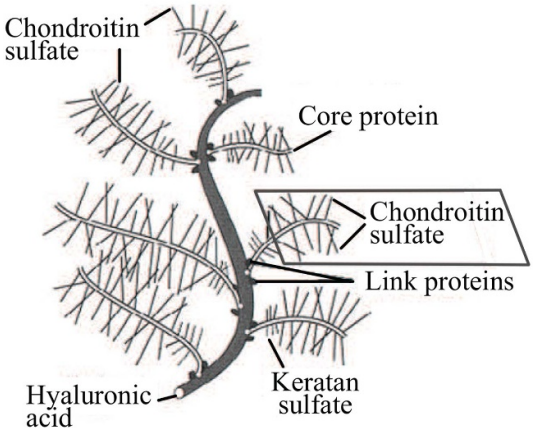

Figure 7. Sketch depicting the structure of a large proteoglycan aggregate consisting of keratan sulfate and chondroitin sulfate attached to the core protein, for example, aggrecan or versican, that are attached to hylauronan molecule via linking proteins; [92] (2012) © Springer-Vertag Wien 2012. With permission of Springer.

binding protein that play an important role in the organization of the ECM [62, 85]; for a recent review see [86].

2.3.3. Proteoglycans

PGs consist of GAGs that are linked to a core protein, and are mainly found in the ECM although some PGs extend across the cell membrane or are directly attached to it by anchors [22, 87]. GAGs are negatively charged and hydrophilic unbranched polysaccharide chains composed of repeating dissacharide units. They can be divided into four groups by their sugars and links between the sugars: (i) chondroitin sulfate and dermatan sulfate, (ii) heparan sulfate, (iii) keratan sulfate and (iv) hyaluronan (also known as hyaluronic acid or hyaluronate). Hyaluronan typically presents as an extremely long chain as it is not linked to any core protein [22].

PGs take part in ECM remodeling and cell adhesion-migration-proliferation by interacting with or functioning as cell surface receptors to growth factors, matrix remodeling enzymes and other ECM components [88]. PGs secreted by vascular endothelial cells can serve as structural organizers of the basal lamina by attaching to the other constituents or contribute to the selective filtration of the basal lamina. SMCs synthesize primarily chondroitin sulfate (large and small), dermatan sulfate (small) based PGs and small amounts of heparan sulfate PGs, which are involved in stabilizing the ECM and regulating cell adhesion-migration-proliferation [89]. Proteoglycans can either promote or prevent these processes depending on the type of cells they are interacting with and the environment [88]. For example, vascular endothelial cells switch to synthesizing chondroitin sulfate/dermatan sulfate-based PG rich ECM from heparan sulfate-based PG rich ECM when cell migration is induced [90]. In addition, the effect of heparan sulfate is inhibitory on the migration of SMCs [91].

PGs found abundantly in the ECM of the vessel wall can be divided into two main categories: (i) large PGs forming large aggregates by interacting with hyaluronan such as versican and aggrecan, see figure 7; (ii) small leucine rich proteoglycans such as decorin, biglycan, fibromodulin, osteoglycin and lumican [24]. Although not arterial wall specific, an extensive list of intracellular, cell-surface, pericellular and extracellular PGs can be found in [87].

Aggrecan consists of keratan sulfate and chondroitin sulfate GAGs attached to the core protein, as depicted in figure 7, and it is the primary load bearing PG of cartilage tissues [93]. Although its role in the aortic wall remains unclear, aggrecan is mainly found in the outer region of the developing wall [24]. A chondroitin sulfated PG prominent in the intima and adventitia of normal blood vessels, versican, forms large aggregates on hylauronan molecule similar to aggrecan and it is mainly secreted by vascular SMCs [94, 95]. Versican can influence phenotype of vascular cells and it is needed for proliferative environment [95]. For example, when proliferation and migration are induced, SMCs secrete versican forming a viscoelastic coat around themselves in which they can change shape to proliferate or migrate [94]. Versican is also shown to bind fibrillin-1 microfibrils [96]. Over-expression of versican isoform V3 by the SMCs is shown to promote elastic fiber assembly mediated by TGF-β pathway [97], and to create an environment resistant to monocyte adhesion [97, 98].

As mentioned before, decorin, biglycan and lumican in the small leucine rich proteoglycan family have been associated with collagen fibrillogenesis, specifically with fibril diameter and organization [24].

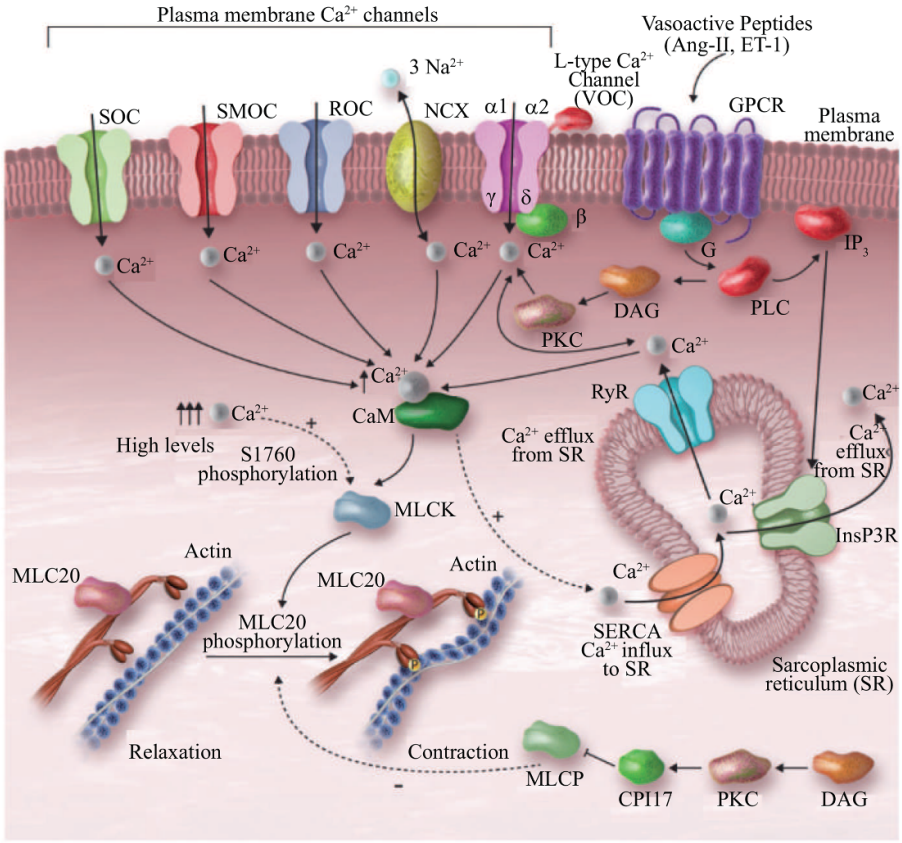

Figure 8. Ca^{2+}-mediated SMC contraction: intracellular Ca^{2+} concentration increases via (i) the influx through store-operated channels (SOC), second messenger-operated channels (SMOC), receptor-operated channels (ROC), Na^{+}-Ca^{2+} exchanger (NCX), or voltage operated channels (VOC) on the cell membrane; or (ii) release from the sarcoplasmic reticulum which can be triggered by IP^{3} binding of vasoactive peptides to G protein-coupled receptors (GPCR). Activation of phospholipase C (PLC) through GPCR can either trigger the production of IP^{3} or the formation of diacylglycerol (DAG). The latter results in activated protein kinase C (PKC), which can either influence the membrane channels to initiate contraction or prevent the activity of myosin light chain phosphatase (MLCP) to keep a contracted state; reproduced from [100]. CC BY 4.0.

Furthermore, decorin and especially biglycan are reported to have high binding affinity for tropoelastin through their core protein instead of their GAG chains, suggesting that they may play a role in elastogenesis [99]. Decorin, found only in the adventitia [24], tends to colocalize with collagen [95], whereas biglycan, found in all three layers [24], tends to localize around SMCs and associates with other ECM components such as elastin [95].

2.4. Smooth muscle cells

As mentioned above, SMCs play an important role on the mechanical state *in vivo*. Additionally, they contribute to the passive mechanical behavior by remodeling the ECM they are embedded in. After introducing SMC contraction mechanisms, we continue with ECM regulation by SMCs. Although there are several pathways which are involved in the ECM regulation, the role of TGF-β signaling is the main focus since it is deeply investigated in relation to TAADs.

2.4.1. Active response

SMC contraction takes place mainly upon intracellular Ca^{2+} concentration increase due to neural, hormonal and local factors. Figure 8 depicts possible Ca^{2+} increase pathways, such as via the release from sarcoplastic reticulum upon activation of G-protein coupled receptor by vasoactive agents as well as influx through store-operated channels, second messenger operated channels, receptor-operated channels, Na^{+}-Ca^{2+} exchanger, and voltage operated channels. Intracellular Ca^{2+} ions then bind to the protein calmodulin (CaM) forming a complex, which in turn binds to and activates myosin light chain kinase (MLCK). Active MLCK phosphorylates myosin light chain (MLC) via ATP hydrolization, exposing the actin binding sites on MLC to form cross-bridges [100].

In addition to an intracellular Ca^{2+} concentration increase, SMC contraction can be modulated by calcium-independent mechanisms which influence the sensitivity of MLC to calcium. These mechanisms,

such as DAG-PLC-PCK and RhoA-Rho kinase pathways, regulate the phosphorylation state of MLC independent of CaM-MLCK signaling by inhibiting the MLC phosphotase (MLCP) activity [100].

Relaxation is mainly initiated by a decrease in intracellular Ca^{2+} which results in dissociated CaM complex and dephosphorylated MLC by MLCP [101]. SMCs, however, do not completely relax but maintain a so-called basal tone. This energy efficient state has low ATP consumption since the cross-bridges are maintained without further MLC phosphorylation [102].

Vasoactive agents triggering SMC contraction such as Angiotensin-II (Ang-II), Endothelin-1 (ET-1) and norepinephrine are known as vasoconstrictors and decrease the lumen diameter, whereas agents inducing relaxation such as NO, L-arginine and histamine are known as vasodilators and increase the lumen diameter. A local increase in the blood flow means a local increase in wall shear stress sensed by the endothelium, and the endothelial cell response is to increase the NO production and decrease the ET-1 production [103]. In the endothelial cell, L-arginine and oxygen are converted by NO synthase into NO and L-citrulline. NO then diffuses from the producer cell to the target cell, and binds to soluble guanyl cyclase on the SMC membrane where it catalyzes intracellular guanosine triphosphate conversion into cyclic guanosine monophosphate, which can induce SMC relaxation via various pathways resulting into two main actions: (i) decreasing the intracellular Ca^{2+} concentration, or (ii) reducing the sensitivity of the contractile unit to Ca^{2+} [104]. SMC relaxation results in vasodilation, i.e. increased diameter and isochorically decreased thickness, decreased shear stress and blood pressure [103, 105].

A local increase in pressure, on the other hand, increases the lumen diameter and decreases the thickness—decreasing the shear stress and increasing the circumferential stress. Endothelial cells respond to the decreased shear stress with decreasing the NO production and increasing ET-1 production [103]. ET-1 is produced by endothelin converting enzyme and it can bind to ET_A receptors on SMCs to act as a vasoconstrictor or they can bind to ET_B receptors on endothelial cells to act as a vasodilator [106]. Vasoconstriction function acts to increase intracellular Ca^{2+} release from the sarcoplasmic reticulum [107], and increased intracellular Ca^{2+} triggers SMC contraction reducing the lumen diameter.

2.4.2. Regulation of the ECM via mechanotransduction

'*Homeostasis requires that intramural cells can sense their chemo-mechanical environment to keep the homeostasis and establish, maintain, repair or remodel the ECM to provide suitable compliance and yet sufficient strength [78].*'

Cell-to-cell and cell-to-ECM interactions are essential for the cells to communicate with their environment. These communications occur via occluding junctions such as tight junctions and communicating junctions such as chemical synapses and gap junctions. Moreover, anchoring junctions connect cells to cells or cells to the ECM. These junctions allow cells to sense their mechanical environment and activate different signaling pathways to modify it. This interaction called mechanotransduction occurs via force-induced conformational or organizational changes in molecules or structures such as ion channels activated with mechanical stimuli, cadherin complexes and integrins [108].

Cell-to-cell anchoring junctions are (i) adherens junctions linking actin filaments and (ii) desmosomes linking intermediate filaments [22]. Figure 9(a) depicts cell-to-cell and cell-to-ECM connections on soft ECM, with a focus on adherens junctions via cadherins, as seen in figure 9(b) [108]. Cell-to-ECM anchoring junctions are (i) focal adhesions and (ii) hemidesmosomes. Figure 9(c) depicts cell-to-cell and cell-to-ECM connections on stiff ECM, with figure 9(d) focusing on the focal adhesions via transmembrane proteins called integrins connecting ECM proteins, i.e. fibronectin, with intracellular actin and microtubule. Hemidesmosome junctions, on the other hand, connect ECM proteins such as laminin with intracellular intermediate filaments [22].

TGF-β is a peptide that controls cell proliferation and differentiation [109]. It is well known for its role in ECM synthesis but it is also involved in ECM degradation by activating metalloproteinases (MMP2 and MMP9) [11, 62]. Latency associated proteins (LAPs) form a complex by binding to TGF-β intracellularly, and TGF-β is inactive in this form. LAP-TGF-β can then bind to latent TGF-β binding proteins creating a structure called large latent complex (LLC), which is secreted and cross-linked to the ECM. LLC can be directly connected with cell membrane integrins and fibronectin network in the ECM, meaning that activation can take place by cell contractile forces transmitted via integrins [110]. In addition, LLC can be sequestered by fibrillin microfibrils and fibulins [110] and fibrillin-1 fragmentation can cause increased TGF-β activity [62]; see, for example, [111] for a more detailed overview of TGF-β and ECM interactions.

Upon release from LLC, active TGF-β binds to the cell surface receptors—TGF-β receptor type I or II—activating canonical or non-canonical pathways. The canonical pathway through SMAD 2/3 cascade is associated with ECM synthesis and stabilization, whereas the non-canonical pathways such as mitogen-associated protein kinase (MAPK) and ERK1/2 cascades are associated with ECM degradation [11]. The end effect of TGF-β signaling highly depends on which receptors are activated, since any of the 7 type I

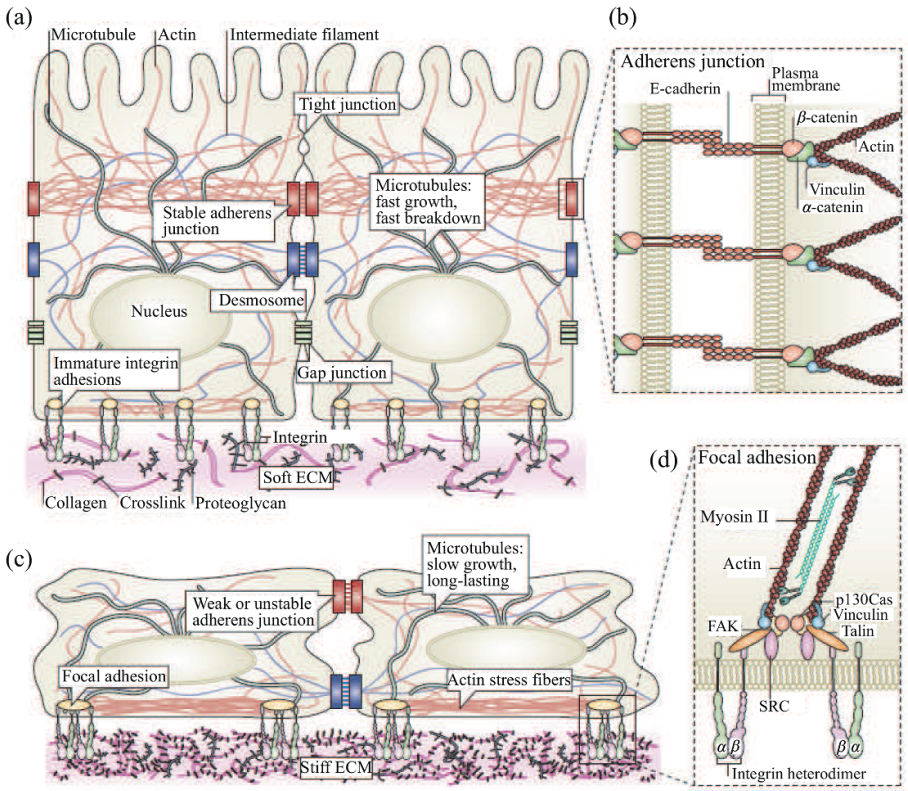

Figure 9. Mechanical network of cells: (a) cell-to-cell and cell-to-ECM interactions on soft ECM; (b) close-up of adherens junctions connecting actin networks of the two cells; (c) cell-to-cell and cell-to-ECM interactions on stiff ECM; (d) close-up of focal adhesions connecting microtubules and actin network of the cell to the ECM via integrins. Bundles of actin fibers are running parallel between adherens junctions and focal adhesions. Increased ECM stiffness due to remodeling can influence the cell shape, the intracellular actin distribution and the organization of cell-to-cell and cell-to-ECM adhesions; [108] copyright © 2011, Springer Nature.

and 5 type II receptors can be involved in the process introducing a high level of complexity [112]. In addition, the combined interactions between the two receptor types may determine the binding of the peptide and the resulting biological activity. The type I receptor plays a role in the induction of genes involved in cell-matrix interactions, whereas the type II receptor is involved in the anti-proliferation activity of TGF-β [113].

Ang-II is a potent vasoconstrictor of the vascular system and its signaling in adults is mostly via the G-protein coupled pathway through Ang-II receptor 1 (AT1), which drives SMC contraction [112]. Multiple levels of interactions exist between TGF-β and Ang-II signaling adding even more complexity to TGF-β signaling, as depicted in Figure 10. For example, activation via AT1 receptors can enhance TGF-β signaling, or it can initiate a mitogen-activated protein kinase (MAPK) cascade leading to ECM degradation independent of TGF-β [11, 62]. AT2 receptors, on the other hand, are expressed during fetal development and pathophysiologies such as hypertension and atherosclerosis [112]. They attenuate both canonical and non-canonical pathways of AT1 induced TGF-β signaling in addition to promoting SMC apoptosis [62]. The reader is referred to [114] for an extensive review on the roles of Ang-II signaling in physiological function and various pathophysiologies.

3. Pathological thoracic aortic wall

Sustained imbalances in the chemical and mechanical signals as well as disturbances to mechanosensing and signal transduction pathways due genetic mutations can disrupt the ECM leading to pathological formations. For example, as mentioned before, endothelial cells respond to locally increased blood flow by inducing dilation of the vessel which decreases the wall shear stress, while increasing the circumferential stress temporarily, both of which go back to normal values when the flow decreases. If the increased flow is sustained this leads to cell-matrix reorganization or ECM turnover to increase the thickness in the dilated state to restore the shear and circumferential stresses back to normal where SMCs proliferate initially, and endothelial cells and fibroblasts initiate growth and remodeling [103]. Since the microstructure of the aorta governs its mechanical behavior, structural changes in the TAADs and selected risk factors influencing the

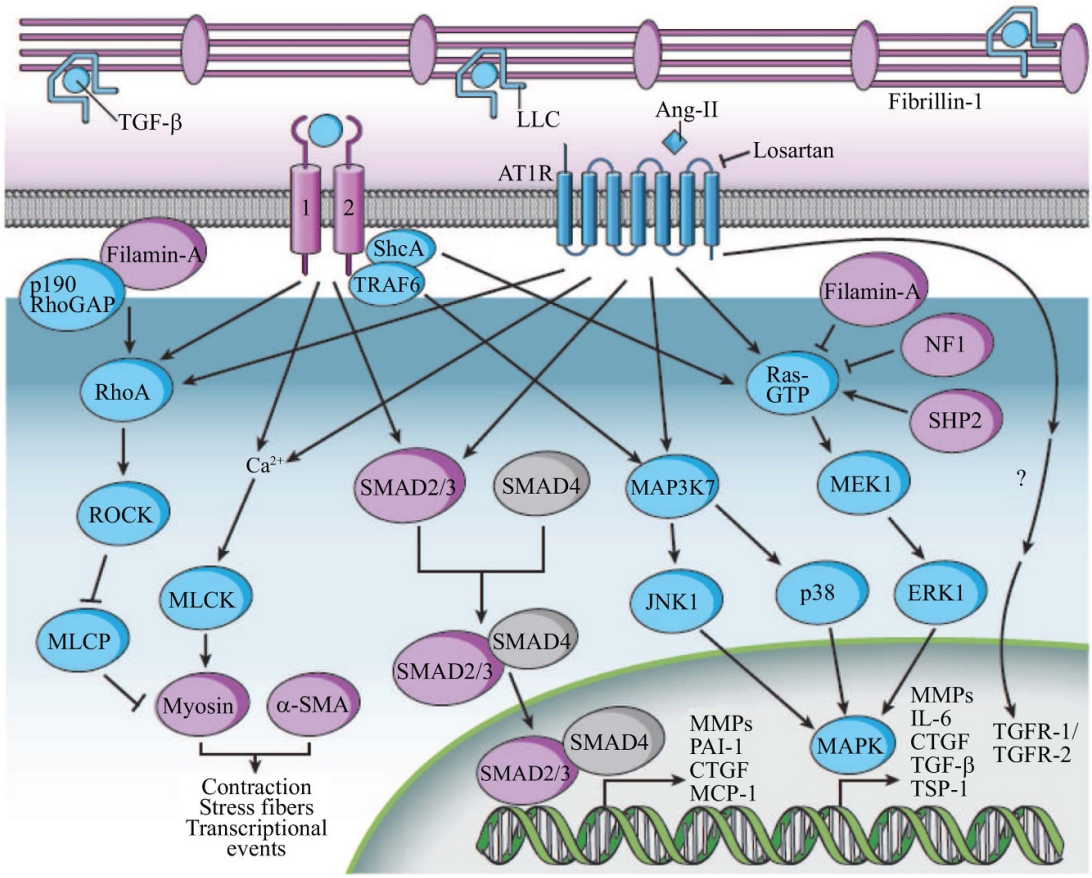

Figure 10. Signaling cascade upon activation of TGF-β R1/2 receptor complex by TGF-β, AT1 receptor by Ang-II, and possible interactions between them. LLC is bound to fibrillin-1 microfibrils and TGF-β is sequestered in the ECM. Upon release, it binds to the TGF-β R1/2 receptor complex and can initiate RhoA, MAPK, SMAD 2/3 cascades, similar to Ang-II upon binding to AT1 receptors. MMPs can be activated downstream to these cascades and promote ECM degradation; [115] copyright © 2011, Springer Nature.

microstructure are summarized before introducing the findings on the mechanical behavior of the diseased thoracic aortic wall.

3.1. Microstructure

Elastic fiber fragmentation accompanied by PG pooling and SMC loss has been reported by several studies for aortic dissections and dissecting aneurysms of the thoracic aorta [62, 117–120]. These pools were observed to span over 3-4 lamellar units and correspond to the areas with SMC loss [121]. Accumulation of GAGs can result in significant stress concentrations and contributions to high intralamellar (Donnan) swelling pressures. This may lead to a rupture of elastic fibers between the elastic laminae and a significant reduction of the radial elastic properties before the event of an aortic dissection [116, 122, 123]; for a schematic drawing of this mechanism see figure 11. Recently, versican and aggreacan were identified as the major components of such accumulations in TAAD patients [124], and these accumulations may result from disturbed proteoglycan production and turnover [125]. Versican accumulation has also been related to atherosclerosis, restenosis and inflammatory processes although exact mechanisms remain unclear [94, 95]. In addition to the three main cell types in the aortic wall, inflammatory cells such as neutrophils, monocytes/macrophages, lymphocytes, adipocytes, mast cells and platelets are also active in the remodeling of the pathological aortic wall [126].

SMCs isolated from the media of aortic dissection and aneurysm patients exhibit reduced expression of contractile proteins [16, 120, 127, 128]. SMCs isolated from dissection patients and cultured *ex vivo* have been reported to show increased proliferation rates [127], whereas SMCs isolated from aneurysm patients were less proliferative compared to controls [128]. Reduced expression of contractile proteins of the SMCs indicate a phenotype switch from contractile to the synthetic type; and such a phenotype switch is typically accompanied by collagen deposition and elastic fiber degradation, as observed for dissection [127, 129, 130] and aneurysm patients [128]. Although the study [120] also found reduced contractile properties for the

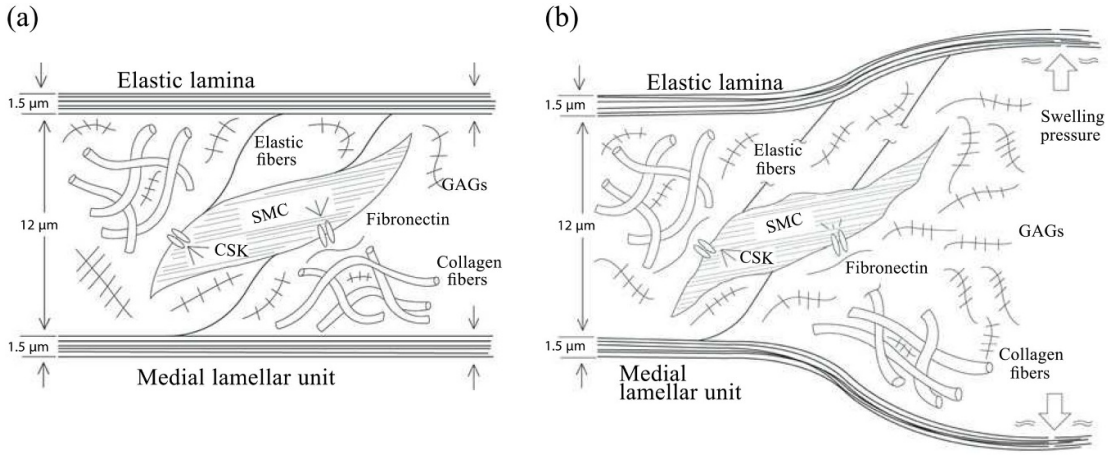

Figure 11. Schematic drawings of aortic medial lamellar units: (a) normal lamellar unit with an embedded SMC, collagen fibers, adhesion molecules such as fibronectin, GAGs and thin elastic fibers providing mechanical connections between the elastic laminae and the SMC. Negatively charged GAGs sequester water and generate a normal intralamellar pressure so that the elastic fibers could be maintained in tension; (b) pathological lamellar unit showing a localized accumulation of GAGs, leading to an increased swelling pressure, which may lead to rupture of the thin elastic fibers and help to separate the elastic laminae. This effect could initiate a local delamination and/or an altered mechanosensitive cellular response which may lead to an aortic dissection; [116] copyright © 2012 S. Karger AG, Basel.

aneuryms and dissections of the ascending aorta, the authors reported disorganized and decreased collagen content instead of an increase in collagen [120, 131].

3.1.1. Risk factors

Hypertension: An experimental acute hypertension model on rats [132], where the authors created a hypertensive environment by inducing coarctation to the mid thoracic aorta, identified an immediate increase in the TGF-β 1 and TGF-β 3 levels as well as an increased cell proliferation for smooth muscle and endothelial cells. TGF-β levels returned back to normal levels after 2 weeks, however cell proliferation was followed by cell apoptosis in 4 weeks. The wall thickness was increased in week 1, and this increase was preserved at weeks 2 and 4, without significant changes in the lumen diameter [132]. These changes in the wall serve to restore the homeostatic stress levels and to preserve them [103]. A sustained increase in circumferential stresses due to a sustained increase in blood pressure can trigger SMCs, endothelial cells, and fibroblasts to remodel the ECM. These actions result in a thickness increase at the constricted state until the stresses are restored to their preferred levels at a preserved lumen diameter. A return to these stress levels would ideally restore NO production levels, however this may not happen in hypertension due to endothelial dysfunction [103].

Bicuspid aortic valve (BAV): BAV is a congenital disorder where the aortic valve has two leaflets instead of three. Although BAV has been associated with mutations in FBN1 [133] and NOTCH1 [134] genes, the mechanisms are not yet fully understood. The study [135] reported that severity of cystic medial necrosis accompanied by elastic fiber fragmentation and changes in the SMC orientation were significantly more in BAV patients than patients with tricuspid aortic valves (TAVs). Even in the absence of genetic mutations, which can also directly influence the structural arrangements in the aorta, altered hemodynamics due to BAV may influence the microstructure of the wall through mechanosensing [136, 137].

Diabetes mellitus (DM): Interestingly, aneurysmatic aortas of DM patients grow at a slower rate [138]; and they appear to be thickened, denser and fibrous [139]. These observations led to the conclusion that DM is regarded as protective against aneurysm formation and rupture in all sections of the aorta. Advanced glycation end products may be responsible for this observation as they are increased in diabetic patients, resulting in proteolysis resistant and stiffer aortic wall. Moreover, the diabetic medications might also contribute to this stability by blocking the production of ECM degrading enzymes and/or their pathways [139]. Diabetic rabbit thoracic aortas contained significantly higher amount of collagen and exhibited significantly higher dissection energy in both directions compared with control, whereas the elastin levels were not significantly different [140].

3.1.2. Extracellular matrix related mutations

FBN1: Mutations in fibrillin-1 gene (FBN1, OMIM® no. 134797) are the major cause of Marfan syndrome, a heritable fibrous connective tissue disorder effecting the microfibril assembly [141, 142]. Histopathology of

patients with manifestations of the cardiovascular system include disruptions to the elastic lamellae, excessive amounts of collagen, PG accumulation, and SMC loss [143]. Mutations can either disrupt the deposition of fibrillin-1 microfibrils or decrease the amount of available fibrillin to form microfibrils [144]. These changes are primarily thought to disrupt TGF-β signaling pathways [143], although other signaling pathways such as epidermal growth factor may also be affected [145]. Aneurysm development can easily be triggered when such disruptions are combined with the hemodynamic loading conditions on the thoracic aorta [78]. For example, the study [146] on mice suggested that aortic dilation in patients with Marfan syndrome is primarily due to failure of microfibrillar array of the adventitia to sustain physiological hemodynamic stress, and the disruptions to the elastic network assembly in the media is secondary. Cultured SMCs obtained from Marfan syndrome mice [147] and from humans [148] were of mixed synthetic-contractile phenotype, and they showed markedly lower force generation capacity, altered mechanosensing as well as over-expression of proliferation markers [147].

COL3A1: COL3A1 gene (OMIM® no. 120180) encodes the α1 chain of type III collagen [142]. Mutations in this gene result in the production of mutant type III procollagen, which was reported to have decreased thermal stability [149]. The structural defects in the procollagens can cause delayed formation as well as destabilization of the triple helix resulting in reduced secretion [150]. Type III rich fibrils with smaller diameter as a result of the mutations were also reported [151]. As opposed to creating structurally altered type III fibrils, in [152] it is suggested that the mutations may rather result in the deficiency of the protein. Normal formation of type III collagen was suggested to be essential for fibrils rich in type I collagen to form also normally [74]. Vascular type of Ehlers-Danlos syndrome (OMIM® no. 130050) is caused by mutations in COL3A1. Patients suffering from this syndrome are prone to spontaneous rupture of large arteries [142] and are known to have fragile arteries [14]. Abnormally low intima-media thickness in elastic arteries of these patients may result in higher wall stresses, which can increase the risk of dissection and rupture [153]. Age related arterial stiffening was attenuated in patients with the Ehlers-Danlos syndrome, and the arterial stiffness in Ehlers-Danlos syndrome patients was significantly lower compared with age-matched control subjects [154].

3.1.3. Smooth muscle cell related mutations

ACTA2: Mutations to the gene encoding smooth muscle aortic α_2 actin (OMIM® no. 102620) create disruptions to the cyclic interactions between α-actin and β-myosin heavy chain [142]. These mutations were found to be responsible for 14% of inherited TAAD [155] and they interfere with actin filament assembly causing decreased SMC contractility [10]. In addition, the increase in adventitial vasa vasorum in cases of mutations in ACTA2 and MYH11 genes suggests possible inflammation processes [156].

MYH11: Mutations to the smooth muscle β-myosin heavy chain coding gene (OMIM® no. 160745) [142] are thought to alter the structure of smooth muscle myosin heavy chain and the assembly of myosin thick filaments [157]. Ang-II and insulin growth factor I were observed to increase in patients with this mutation, however it remains unclear how [158]. The histological and immunohistochemical investigations of the affected aortic tissue samples showed SMC, elastic fiber and collagen loss [157]. Interestingly, the study [159] did not find marked changes in the microstructure between the control and the mutant mouse model under normotensive or hypertensive conditions suggesting a structural adaptation. However, outliers (in terms of mechanics) in the hypertensive mutant group showed local GAG pooling and intralamellar delaminations [159].

MYLK: This gene encodes the myosin light chain kinase enzyme (OMIM® no. 600922), which positively regulates muscle contraction [142]. The mutations cause loss of enzyme function by altered CaM binding [160]. Mice with knockdown MYLK gene showed PG pooling in the media consistent with increased expressions of lumican and decorin, however, there was no elastic fiber degradation [160]. These authors also reported an increase in type III collagen in the adventitia.

3.1.4. TGF-β related mutations

Although many studies report an increase in TGF-β levels in aneurysms and dissections, the role of this molecule in TAAD remains highly controversial [161]. Different isoforms of TGF-β can substitute for each other or they can activate a different signaling pathway, and loss-of-function mutations to a certain TGF-β isoform can manifest as, seemingly contradictory, increases in TGF-β activity [162–164]. Direct mutations of the TGF-β signaling pathways are associated with different phenotypes of Loeys-Dietz syndrome (LDS). Three of these phenotypes (LDS1, LDS2, LDS4) are related to the genes explained below, whereas LDS3 and LDS5 are related to the mutations in SMAD3 (OMIM® no. 603106) and TGFB3 (OMIM® no. 190230) genes, respectively [142].

TGFB2: Mutations to TGFB2 gene (OMIM® no. 190220) cause LDS4 [142]. Ascending aortas of mice with this mutation were comparatively small and thin walled [165]. In addition, mice that have only a single

functioning copy of this gene (haploinsufficient) have aortic root aneurysms, and an increase in canonical and non-canonical TFG-β signaling [163]. Histological sections of the aortas from mice with heterozygous mutations showed elastic fiber fragmentation and collagen deposition [163].
TGFBR1: This gene encodes the serine/threonine kinase receptor type I for TGF-β (OMIM® no. 190181) [142]. Mutations are reported to result in LDS1 and to cause activation of the TGF-β signaling pathway [109]. The study [166] reported severe defects in vascular development and in the absence of circulating red blood cells, and the mice embryos died prematurely.
TGFBR2: This gene encodes the serine/threonine kinase receptor type II for TGF-β (OMIM® no. 190182) and causes LDS2 [142]. The study [167] reported loss of elastin content as well as disrupted organization of elastic fibers in the aortic media. Furthermore, the authors suggested that these characteristics were due to the disruptions in elastogenesis rather than secondary destruction of the elastic fibers. In addition, the aortas from patients with TGFBR2 mutation accompanied by Marfan syndrome presented even higher collagen content, compared with no additional Marfan syndrome.

3.2. Mechanical behavior

Aneurysmatic tissue was reported to behave significantly stiffer compared with controls in the circumferential [168] but also in the longitudinal direction [169] by comparing the peak elastic modulus from uniaxial tensile tests. On the other hand, biaxial tensile test data at physiological load levels [170] showed aneurysmatic tissue to be stiffer than control tissues in both directions. The study [171] documented that strips from ascending aneurysm and control tissue are significantly stiffer and stronger in the circumferential direction compared with the longitudinal direction. The authors also observed that aneurysms did not cause wall weakening, but the strips from aneurysmatic tissues were stiffer and less extensible than the strips from control tissue. Furthermore, the experimental study [172] reported that the dissected tissues were significantly stiffer in their collagen dominated response in the circumferential direction compared with control tissues in [173]. Unfortunately, none of the above mentioned biomechanical studies quantified the collagen architecture in terms of fiber orientation, which could shed more light on the findings regarding anisotropy and heterogeneity of the pathological and control aortic tissues. Regardless, the studies [171, 173, 174] reported reduced elastin accompanied with unchanged collagen content.

The aneurysmatic aortic wall from older patients were stiffer compared with younger patients [175], similar to the healthy aortic wall. In contrast, the study [172] found no influence of age on the mechanical behavior of dissected tissues. The degree of collagen fiber alignment increased with age and caused a stiffening that was primarily seen in the abdominal aorta, although collagen fibers in the intact wall were predominantly aligned circumferentially in all aortic locations for all age groups [176].

Uniaxial tensile tests were used to characterize the regional and directional differences by comparing the low and high elastic moduli (related to initial compliant region and stiff region of the tensile curve, respectively) of ascending aortic aneurysms in [177]. The authors reported a significantly higher maximum elastic modulus for specimens from both posterior and anterior regions in the circumferential than in the longitudinal direction, whereas there was no significant differences in the low elastic modulus. Transition stretch was significantly higher in the circumferential compared with the longitudinal specimens from both anterior and posterior regions. Anisotropy of aortic tissues from ascending aneurysms has been confirmed by other studies using uniaxial tests in circumferential and longitudinal directions [168, 178–180]. In contrast, other studies did not find an evidence for anisotropy of aneurysmatic tissues under uniaxial loading in circumferential and longitudinal directions [169] and biaxial loading [173], and of dissected tissue under biaxial loading [172].

Layer-specific uniaxial tensile tests on human ascending aortic aneurysms in [181] demonstrated regional heterogeneities in tissue stiffness for all layers. Highest and lowest values for the media and the adventitia observed for the right and left lateral regions, respectively, similar to the observations for the intact wall [168]. The authors reported a significantly higher stiffness in circumferential strips compared with longitudinal strips for both medial and adventitial layers [181].

Opening angles of aneurysmatic ascending aortas were significantly increased with age [175, 182], while opening angles decreased significantly with increasing distance from the aortic root [182]. They were smaller in the control group compared with the TAV group, and with the BAV group lying in between, see figure 12(a). The circumferential residual stretch difference decreased in a similar fashion [182], see figure 12(b). Furthermore, the author also reported that opening angles increased significantly with atherosclerotic plaque buildup, but for a given amount of plaque they were similar between the three groups. Strips of aneurysmatic (BAV and TAV) and control thoracic aortas exhibited greater tensile circumferential residual stretches in the media than in the adventitia, whereas the opposite was observed in the longitudinal direction albeit still tensile. The residual deformations did not show marked differences around the circumference of the wall for the media and the adventitia. However, the intima was under smaller residual

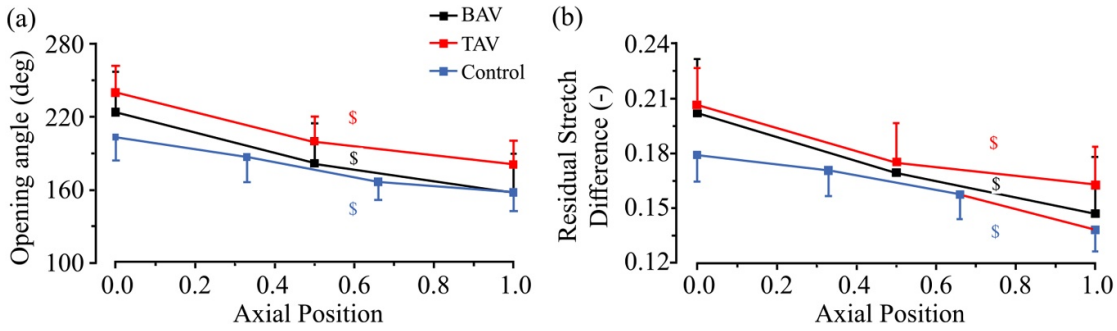

Figure 12. (a) Opening angle and (b) circumferential residual stretch difference versus the (non-dimensional) axial location in the ascending aorta (axial location of $x/100$ means that the specimen was located x% of the total ascending aortic length away from the root). Data are provided as mean values ± SE. The symbol $ characterizes significant regional differences. BAV = bicuspid aortic valve patient, TAV = tricuspid aortic valve patient; reprinted from [182], Copyright (2015), with permission from Elsevier.

compression circumferentially in the posterior quadrant and longitudinally in the right lateral quadrant compared with the other quadrants [182].

The study [183] investigated the role of fibulin-5 deficiency, a mutation that yields to severe arterial elastopathy but not to aneurysms or dissections. The authors reported marked structural stiffening manifesting as reduced distensibility and extensibility that contributed to a decreased elastic energy storage and an increased energy dissipation in the central arteries of fibulin-5 deficient mice, changes being most prominent in the ascending and the abdominal aorta. In line with these findings, the results in [184] also showed that not only elastin, but properly assembled and cross-linked elastic fibers are responsible for low energy loss in the aorta.

MYH11 mutation leads to an early and severe decrease in the elasticity of the aorta [157]. The change in the structural stiffness between control and mutant mouse aortas under normotensive or hypertensive conditions was not as dramatic in [159] as that reported for humans [157]. The MYH11 mutant mice under normotensive conditions showed, although not significantly, decreased elastic-energy storage and increased energy dissipation in response to pressurization, suggesting also mechanical adaptivity, and they did not develop aneurysms or dissections [159]. The authors reported that most specimens in the hypertensive mutant group also showed structural and mechanical adaptivity, however, as mentioned in section 3.1.3, those showed delaminations were mechanical outliers and they exhibited marked decrease in energy storage.

The study [185] found a significantly higher energy loss in the longitudinal direction, defined as the hysteresis divided by the total strain energy, under biaxial testing in samples from aneurysmatic walls compared with control tissues. The authors reported that this energy loss was correlated with aortic size and associated with medial degeneration and increased collagen to elastin ratio. In a follow-up study [186], they reported a higher energy loss in the circumferential than in the longitudinal direction for both control and aneurysmatic aortas pointing to anisotropy. However, the degree of anisotropy was different for the individual samples. A tendency towards energy loss isotropy was observed for the aortas with a high collagen to elastin ratios. This loss of anisotropy was the mechanical demonstration of severe medial degeneration, which was characterized by elastic fiber fragmentation.

4. Summary and concluding remarks

Thoracic aneurysms and dissections, the two pathologies that represent the main subject herein, are fatal in case of rupture and they are characterized by changes to the aortic wall microstructure. Hence, there is a pressing need to improve our understanding of the tissue remodeling and failure.

Adverse events such as dissection or rupture of the TAA can occur at any size [13, 21, 187, 188] and current guidelines mainly based on the critical diameter are not able to prevent 60% these events [187]. An increased aortic diameter is associated with decreased distensibility. The study [189] describes that the ascending aorta starts acting as a rigid tube at a diameter around 6 cm where it cannot stretch anymore to assist the systolic cardiac load. In other words, as the aorta grows, its elastic energy storage capability becomes significantly impaired resulting in full cardiac load being transferred to wall stress. Moreover, an increased wall stiffness is associated with higher aortic root growth rates. Although treatments aiming to decrease the stiffness may slow down the growth, they may also increase the risk of adverse events since fibrosis could be preventive against them [161].

In section 2 we have seen that the ability to communicate between the intramural cells and the ECM is essential to keep a homeostasis, and there are numerous instances where things can go wrong. Intramural cells, and consequently the aortic wall, may be able to adapt to mutations and disturbances keeping the organ functional. However, sustained disturbances and/or failure to adapt will result in pathological formations, as depicted in section 3.

The aortic wall consists of collagen fibers embedded in an elastic matrix, and it can be seen and modeled as a fiber-reinforced composite material. It is known that the microstructure, especially the mean orientation of collagen fibers and their dispersion around this orientation, determines the stress-strain response of the tissue. However, biochemomechanical roles of various other structural components remain to be identified. For example, the role of PGs in TAAD pathophysiology remains largely unclear and complex roles they play in different stages of atherosclerotic formations [95], seem to be crucial especially in regard to signaling pathways.

From a biomechanical point of view, dissection and rupture occur when the stresses acting on the wall exceed the wall strength (for a recent review on aortic wall failure see [190]). Considering the multiaxial loading state *in vivo*, one needs to identify wall stresses and wall strength under different loading modes. In addition to the challenging task of reliable estimation of strength and *in vivo* loading conditions, an application to the clinic requires the ability to predict the response of the organ to a variety of biological, chemical and mechanical stimuli over various time scales as they may promote pathological growth and remodeling.

Based on the information presented hitherto, it is clear that a systematic quantification of the changes in the microstructure in healthy and diseased thoracic aortas are necessary to reliably estimate their influence on the mechanical response. There is a pressing need for additional mechanical and microstructural data to better inform material modeling.

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