Characterizing the mechanical properties of the aortic wall

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Abstract

Characterizing the physical properties of the aortic wall is essential to understanding the causes of cardiovascular diseases, such as aneurysms. Modelling compliant, anisotropic, multilayered tubes such as the aorta has proven to be a challenge. In vitro studies of the mechanical properties of arteries incorporate a variety of testing methods; however, the majority of these tests fail to replicate the complex, transmural loading conditions arising from pulsatile flow. These methods include typical tensile tests, both in uniaxial and biaxial set-ups, bulge inflation tests and extension-inflation tests. Bulge-inflation tests grant material information in response to biaxial loading but still do not mimic proper cylindrical loading conditions. Extension-inflation tests capture the cylindrical loading but have only been performed with static pressurization and with rigid boundary conditions in effect. This review aims to present the current state of the biomechanical characterization of arterial walls, particularly the aorta, through discussion of testing methods and their findings. We emphasize literature that focuses on prediction of aneurysm rupture risk. Moreover, overarching concepts such as histological effects, age dependent effects, segmental effects, hemodynamic effects, viscoelastic modelling and torsion will be briefly explored. An understanding of the current limitations of testing will hopefully lead to the development of more robust in vitro test methods that will further elucidate the relationship between changing vessel wall mechanics and cardiovascular disease.

Keywords: Aortic aneurysm, biomechanical testing, aortic stiffness, aortic rupture

INTRODUCTION

Aortic aneurysm can be a life-threatening condition, representing a serious mortality risk of 80% if rupture occurs [1]. There is a significant decline in mortality risk if aneurysms are electively treated with aortic
replacements when compared to non-elective replacements\cite{2}. Therefore, early predictions of aneurysm rupture risk can be of great benefit to patients. Currently, an aortic aneurysm diameter greater than 5.5 cm is an indication for intervention. However, in patients with connective tissue disorders, where structural changes in the aortic wall are well defined, a much lower threshold of dilatation is warranted for intervention; in Marfan Syndrome, a diameter of greater than 5.0 cm is an indication for surgery with a lower threshold of 4.5 cm in the presence of further risk factors\cite{3}.

Aneurysm diameter and growth may not accurately predict the risk of aneurysm rupture\cite{4} and might lead to undertreatment of those who have other contributing factors such as aortic stiffness or a connective tissue disorder. Alternatively, overtreatment is also a possibility: Trabelsi et al.\cite{5} found that even at a diameter of 6 cm only 31% of patients with aneurysms developed complications. Moreover, in the general population, there is a large number of individuals with aortic diameters between 5.0 and 5.5 cm whose risk of complications are not well understood\cite{6}. The Laplace Law is the theoretical basis of the diameter guidelines; however, this law is valid for simple spheres and uniform cylinders and might not adequately appreciate the complex geometrical nature of the native vessels\cite{1}.

The biomechanical analysis of diseased aortas could therefore represent an intriguing opportunity to further elucidate the mechanisms leading to rupture even in the absence of connective tissue disorder. It has long been recognized that a blood vessel cannot be considered as a passive conduit for blood flow. Rather, a blood vessel is a continuously adapting, dynamic element with the purpose of maintaining optimal function in response to changing hemodynamic conditions\cite{7}. Therefore, a more thorough understanding of the biological composition and biomechanics of the vascular system is warranted. There is a need for experimental data with an effort to determine the response of the aorta to a pulsatile waveform. Biological tissues, though subject to conservation of mass, momentum and energy, have unique constitutive equations that differentiate them from inorganic materials, and thus make them harder to characterize. This review aims to present, in brief, the current state of the biomechanical characterization of arterial walls, particularly the aorta, through discussion of testing methods and their findings. Moreover, overarching concepts such as histological effects, age dependent effects, segmental effects, hemodynamic effects, viscoelastic modelling and torsion will be briefly explored.

**CURRENT TESTING METHODS**

**Uniaxial tensile test**

Uniaxial tensile tests have been widely used to test biological tissues *in vitro* due to their simplicity and ability to give precise information regarding local properties of soft tissues. While extending a piece of the sample (either rectangular in shape, or dog-bone shaped), the displacement and resulting force are recorded until fracture [Figure 1A]. This data can be used to obtain a variety of stress-strain relations, and depending on the constitutive model chosen, authors may make use of different stress and strain parameters outlined throughout Continuum Mechanics such as Cauchy stress, engineering stress, Second Piola-Kirchoff stress, Green strain, true strain, and engineering strain.

With uniaxial tensile testing, one can obtain the ultimate tensile strength (strength most often recorded at failure), the yield strength, as well as the strain at failure. A single value of Young’s modulus, which is useful for defining non-biological materials, cannot be adequately used for blood vessels since they are characterized by a non-linear elastic behaviour. A common method to define this parameter for biological tissues is to calculate the modulus incrementally and report it for a specified range of stress. Additionally, most authors make use of the maximum tangential modulus to describe vessel stiffness and make succinct comparisons between intra-study specimens. Currently there is no standard for testing protocol, and variations in experimental parameters such as the force range and number of cycles for preconditioning,
loading or strain rate, or the strain measure used to calculate elasticity parameters, make lateral comparisons between papers difficult at best\cite{4,8}.

A focus of uniaxial testing on the aneurysmal aorta has been considering the region- and layer-dependent variations of the wall behaviour. Iliopoulos et al.\cite{9} first showed that the ascending thoracic aortic aneurysm (ATAA) exhibits heterogeneity between the anterior, posterior, right and left lateral regions. Note that the following studies presented in this section divided specimens into these same four regions. The data from the circumferential direction suggested this direction to be stiffer than the longitudinal direction at physiological and high stresses. While no differences in the peak elastic modulus were observed between the four regions in the circumferential direction, the longitudinal direction results indicated that the anterior region was significantly less stiff than the other regions. The results showed that no correlation existed between failure stress and the diameter of the whole ATAA\cite{9}. The layer-specific differences of the tunica intima, media, and adventitia from uniaxial data of the ATAA were documented by Sokolis et al.\cite{10}. In general, the circumferential stiffness was recorded as higher than the longitudinal direction in the adventitia and media, but not the intima, though all layers had a higher failure stress in the circumferential direction. This is true for all four regions (previously introduced) of the media, but only for the anterior and posterior regions of the adventitia. This study also brought up the important concern that the adhesive connections between the three layers needs to be considered when drawing conclusions regarding the overall mechanical

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{schematics.png}
\caption{Schematics of the mechanical tests described for aortic mechanics where A: shows a uniaxial test; B: biaxial testing; C: the bulge-inflation test; D: depicts inflation-extension testing; E: opening angle testing: Upon cutting an intact circumferential segment of an artery in an unloaded state (described by radius, \(r\)), an expansion of the segment is observed over time, leading to an equilibrium zero-stress state (described by radius, \(R\), and opening angle, \(\theta\)). The residual strains can be obtained from comparison between the two states; F: nano-indentation test: an indenter of known material properties is pressed into the tissue with a known loading pattern, after which the area of the indentation is observed, and the hardness of the material can be calculated.}
\end{figure}
properties of the wall from those of the individual layer behaviour. Khanafer et al.\cite{11} performed uniaxial tests on the ATAA and highlighted the importance of the elastic modulus in assessing risk of aneurysm rupture. Sassani et al.\cite{12} examined the regional uniaxial response (collecting bi-dimensional strain data) of the intimal, medial, and adventitial layers of the aneurysmal ascending aorta in the circumferential and longitudinal directions, again separating the specimens into four regions. A decoupled microstructure-based formulation, resulting in a reduced two-fiber model, was used to describe the uniaxial behaviour in either direction, concluding that material parameters are highly dependent on differences within the underlying wall composition. Further studies on these histological effects are presented under the section titled Factors Affecting Arterial Mechanics.

**Biaxial tensile test**
While uniaxial tests describe failure properties of vessel walls adequately, as shown by the studies cited in the previous section, characterising loading response along a singular axis does not reflect \textit{in vivo} conditions, where the blood vessel wall is subject to multi-axial stresses. Guinea et al.\cite{13} performed uniaxial tests on healthy thoracic aortas, with samples obtained from persons deceased from non-cardiovascular causes. They acknowledged the limitations of the uniaxial test, stating that it poorly reproduces the complex loading conditions seen at the physiological level, which are better described by biaxial testing. In addition, uniaxial testing does not provide a true understanding of the anisotropic properties of the vessel wall, as the circumferential and axial test strip come from different locations. Biaxial testing is done with a square sample, which is placed in a loading rig with four arms, generally by means of hooks, at 90 degrees to each other [Figure 1B]. A significant volume of literature has focused on biaxial testing of both healthy and diseased tissue\cite{14-18}. Alreshidan et al.\cite{19} used biaxial testing on resected ascending aortic tissue to compare to and validate the use of \textit{in vivo} speckle tracking transesophageal echocardiography to estimate aortic stiffness to better stratify the risk of aortic aneurysm rupture. The advancement of \textit{in vivo} imaging techniques gives rise to more reliable data; however, the behaviour of the aorta with these tests is limited to patient-specific physiological conditions. In addition, \textit{in vivo} testing presents multivariate data, as it is not possible to disregard other non-loading effects, such as the effect of surrounding perivascular adipose tissue. Drawbacks of biaxial testing arise from the attachment method, i.e., the failure of the tissue usually occurs at the attachment location and rupture is also influenced by the components used to hold the tissue\cite{20}. In addition, testing samples of various shapes does not allow for accurate comparison since the geometry, in addition to the clamping method, can alter the mechanical properties observed, as was described by Waldman et al.\cite{21}.

**Bulge inflation test**
The bulge inflation test, also referred to as the membrane bulge test, gives information on biaxial behaviour, and was used most recognizably in the work by Mohan and Melvin\cite{22} on healthy human thoracic aorta specimens. This test involves using a square specimen, which is secured in the inflation device through an airtight seal [Figure 1C]. A fluid, generally water, is released at a specified rate, and the expansion of the specimen is tracked optically with complex digital imaging techniques to obtain the strain field. This technique has also been applied to analyze the mechanical behaviour of aneurysms\cite{5,23,24}. Romo et al.\cite{23} utilized the bulge inflation test on the ascending aorta to observe the most likely location of aneurysmal rupture many stages of deformation before the rupture took place by noting the region that had the most amount of localized thinning. They created local thickness over pressure maps to predict the site of aneurysm rupture and they posited that the site of rupture mostly occurs not at the location of maximum stress but at the location of greatest wall weakening.

**Inflation-extension test**
An inflation-extension test differs from the aforementioned techniques in that it replicates the \textit{in vivo} cylindrical loading scenario of a blood vessel. In general, an intact portion of a blood vessel is extended
by some means to replicate axial loading, and fluid is run through the conduit to enact circumferential loading [Figure 1D]. Optical methods may be used to track displacement and force of pre-set markers on the blood vessel[25,26]. Courtial et al. [27] present, in detail, an inflation device that was coupled with non-invasive imaging techniques such as ultrasound, in order to identify the parameters of a silicone tube based hyperviscoelastic model that represented the native aorta. They found that the inflation-extension test was adequate in validating this model. The inflation-extension property of the aorta was further tested by Horny et al. [28] in an analytical simulation, using parameters from autopsy measurements. They established that while axial pre-stretch, which allows the aorta to minimize deformation during systole and diastole, decreases with age, the prespecified value of axial pre-stretch can still have a significant effect on the mechanical properties of the vessel wall. Inflation-extensions tests are valuable for understanding the operating mode of arteries. However, inverse analysis and simplification is typically required to find the stress-strain relationship from the pressure-diameter measurements[13].

Opening angle test

Residual stresses are present in the circumferential direction of the arterial wall, and this can be visually shown by cutting open a ring segment of an artery along the axial direction [25,26]. Subsequently the opening angle formed by the specimen may be optically measured until the cut section stabilizes [Figure 1E]. Accounting for these residual stresses, which have been shown to be present in the axial direction as well[29], leads to a significantly lower circumferential stress gradient along the thickness of the vessel[30]. This observation is better explained when considering the circumferential residual strains, wherein accounting for these strains works to decrease the stretch ratio at the inner surface, while increasing that of the outer surface[31,32]. In essence, to account for residual stresses, “state 0” (stress-free state) is taken as the reference configuration, rather than “state 1” (unloaded state - outlined in Ref. [30]). Moreover, the presence of residual stresses in arteries results in a more uniform stress distribution throughout the vessel wall[31,34]. Zheng and Ren[35] further explored the effects of three-dimensional residual stresses in each of the three arterial layers. They showed that the residual circumferential stress is compressive within the intima, while tensile in the remaining two layers, and that the bending of the media in the longitudinal direction noticeably affects the mechanical behavior of the arterial wall. Cardamone et al. [36] attribute the origin of this residual stress to non-uniform growth and remodelling as well as temporo-spatial variances in wall components.

Sokolis et al. [37] examined the regional distribution of circumferential residual strains in the human aorta according to age and gender to gain a more detailed understanding of the zero-stress and no-load states of the human aorta. The opening angle measurement, which characterizes the circumferential residual strain, was highest in the aortic arch and declined in the descending aorta. Opening angle measurements for aneurysmal tissue are difficult to obtain due to the irregular shape of the diseased vessel wall. Sokolis [38] discussed the residual stresses within the ATAA in great depth, presenting several important conclusions regarding the vessel wall behaviour: (1) change in the wall composition, mainly the decreased presence of elastin within the aneurysmal vessel wall, results in greater viscoelastic behaviour; (2) residual strains vary along the circumferential direction within each of the three layers of the wall; and (3) analysing the residual stresses of the layers individually reveals that the intima is held in compression, while the media is in tension. Contrary to previous studies performed on the abdominal aorta, the adventitia was shown to be under compression in the circumferential direction, though still under tension longitudinally. Higher opening angles were measured in the aneurysmal tissue and it was postulated that this might be a compensatory mechanism to increase the vessel’s resistance to dissection [39]. This is perhaps similar to a compensatory increase in opening angle measurements with increased stiffness of the aorta with age. Most recently, Sokolis et al. [40] employed opening angle testing to study the axial residual strain variations in the human aorta according to age and gender. Interestingly, they found that the axial opening angle and residual stress decreased with age as compared to the circumferential residual strain which had increased with age.
Nanoindentation test
Nanoindentation tests may be used to characterize local deformation and properties in multilayer materials [Figure 1F]. Though it is known that the artery is made up of three distinct layers, a large portion of the aorta's biomechanical modelling describe it as a single, homogenous layered vessel. That being said, experimental results regarding the behaviour of the individual layers has been performed on ATAA tissue in detail, and strain-energy-functions were fit to the data to describe the behaviour. Reiterating the discussion from Uniaxial Testing, it was shown that the intima was the weakest of the three layers, with the adventitia exhibiting the highest failure stresses and resistance to excessive deformation, preventing rupture. Sokolis et al. [10] revealed that the behaviour of the intact aneurysmal wall was most similar to the intima and media layers, however, drawing inferences regarding the overall layered vessel wall behaviour remains a challenge due to the complex attachment and interaction between layers.

Hemmasizadeh et al. [41] introduced a custom nanoindentation technique showing that the inner layer is more compliant than the outer layer and sustains higher strains. Therefore, rupture of the aorta travels outwards from the inner layer. This is verified by Manopoulos et al. [42] by uniaxial tests performed on ascending thoracic aortic dissected tissue to understand the failure properties for the vessel wall as divided into an inner (intima-media) and outer (media-adventitia) layer. They found that the inner layer displayed a significantly lower strength than the outer layer, in addition to a sharp decline of stress following rupture, unlike the outer layer which exhibited a slow decrease to zero stress. Interestingly, the relative strengths of the two layers differed significantly in magnitude from what has previously been reported on the individual layers [10,42]. The outer layer was shown to be stronger than the adventitia alone, and in comparing the properties of the origin of inner layer failure to distal locations, it was evident that dissection occurred where the layer was thinner and exhibited increased stiffness. These results offer valuable insight as to why the specimens studied [42] dissected, rather than undergoing full rupture.

FACTORS AFFECTING ARTERIAL MECHANICS
Histological effects
Soft tissues, including blood vessels, contain collagen, elastin, and ground substance. The relative amount, density, and arrangement of these three constituents greatly affect the mechanical response of the tissue [43,44]. Therefore, it is important to consider histological composition when characterizing the physical properties of the aortic wall. Distribution of elastic fibres in soft tissues and its relation to mechanical properties have been studied extensively [43,45]. Bellini et al. [18] better defined the mechanical environments of the media and adventitia layers within the arterial wall by highlighting the roles that smooth muscle cells and fibroblasts play in arterial homeostasis. Taghizadeh et al. [46] evaluated the mechanical properties of the aortic wall while accounting for the lamellar structure within the media layer. Cardamone et al. [36] confirmed that elastin contributes significantly to the shortening of arteries observed upon cutting along the circumferential direction (residual stresses), and to the opening angle phenomenon. Holzapfel et al. [47] combined histological data with computational modelling to create a general constitutive model of the anisotropic collagen fibre dispersion in arterial walls. In terms of aneurysmal tissue, studies focusing on the ascending aorta report reduced levels of elastin, but normal levels of collagen, throughout the vessel wall when compared to a healthy aorta [48,49]. Aneurysm development in the ascending aorta has been shown to be associated with higher stiffness of the wall, resulting in increased wall stresses, but not leading to a weakening of the wall for age-matched subjects [46].

Segmental effects
There are segmental differences in the structure and mechanical properties of the aorta, and these are dependent on the magnitude of stress that the wall is subjected to regularly under normal conditions. Schrieff et al. [50] incorporated the themes of segmental and histological analysis to study the layer specific distribution and orientation of collagen fibres in the abdominal and thoracic aorta. They concluded that
there were distinct fibre families, directions and dispersion present in the three arterial layers; these variations between the layers underlie their different mechanical and functional properties. Sassani et al.\cite{12} utilized a four fiber microstructure based model to characterize region and layer-specific material properties in ascending aortic aneurysmal tissue samples; their novel hypothesis that the fibers are able to support compressive forces provides further insight into the role of elastin and collagen in withstanding mechanical loads. The aortic wall shows an increase in viscoelastic creep further from the aortic root, which can be attributed to a decrease in elastin content. Irregular variability in the opening angle was observed over the length of the vessel, the pattern of which was determined to be similar across both young (less than 40 years of age) and old subjects\cite{37}. Haskett et al.\cite{14} delved into the critical importance of gaining a better structural model of the aorta through quantification of microstructural and mechanical changes. Specifically, they looked at anisotropy and extra-cellular matrix microstructure changes as a function of location and age of the aorta.

**Age-dependent effects**

Another concept of interest is the age-dependent elastic behaviour of the arterial wall which is closely related to the histological compositional changes that occur with age. These alterations with age are explored by Maceri et al.\cite{51}; they introduce a multiscale mechanical model that accounts for nanoscale effects of cross-link stretching, as well as micro- and macroscale mechanisms. This model further supports experimental evidence regarding the histological alterations in the aortic wall that occur with age, showing evidence between the influence of cross-link density and stiffness on the elastic modulus. With age, there is an increase in aortic diameter, thickness, elastin stiffness and collagen cross links and there is a decrease in collagen amount, collagen fiber radius and waviness. Carallo et al.\cite{52} concur and describe the effects of ageing as a change in the arterial wall with an increased collagen presence and reduced elastin function, leading to a larger and stiffer vessel. Moreover, this increase in aortic stiffness and thickness is greatest circumferentially\cite{14}.

Cavalcante et al.\cite{53} present a comprehensive review on arterial stiffness associated with age and, in particular, the acceleration of aortic stiffness due to hypertension. They identify arterial stiffness as an important prognostic factor and emphasize its deleterious effect on the Windkessel function of the aorta. Moreover, they identify pharmacological and non pharmacological therapies targeted against increased aortic stiffness. This further highlights the importance of identifying contributing factors that affect vessel function so that more targeted therapies can be employed. A monotonic decrease in circumferential and axial tensile strength of the arterial wall with age is evident as well\cite{13,54,55}. Guinea et al.\cite{13} utilized uniaxial tensile tests on donor thoracic aortas and found that the tensile strength of the thoracic aorta falls rapidly after age 30 and Morrison et al.\cite{55} found that circumferential and longitudinal strain decreased by 50% with increasing age (patients with a mean age of 68 compared to patients with a mean age of 41). Deveja et al.\cite{56} found a negative correlation between failure stress and stretch with increasing age in all three layers of the ascending aorta and a similar correlation was found by Iliopoulos et al.\cite{57} in tissue samples of patients with Sinus of Valsalva aneurysms. Tracy and Eigenbrodt\cite{58} found that a disproportionate increase in vessel wall thickness with age causes a deviation from the Laplace law: they introduced age specific constants into the Laplace equation to make their data conform to Laplace expectations. This further highlights the need for a more comprehensive guideline to assess aneurysm rupture risk, especially in an ageing population, than the current diameter-based guidelines which were formed on the basis of the Laplace Law.

**Hemodynamic effects**

Hemodynamics is of particular interest as a potential factor in arterial disease formation and progression. Dynamic in vitro tests could show the effect of flow on the healthy arterial structure and subsequent disease formation while still allowing for control of the loading conditions and isolating mechanical effects. Pulsatile arterial hemodynamics has been explored in numerous studies\cite{52,59,60}. The scope of this
review is not intended to cover studies on hemodynamics and associated arterial mechanics; however, to fully understand the complexity of characterizing the vasculature wall and the mechanisms that may lead to cardiovascular complications, the fluid-structure interactions - for example, wave propagation in arterial walls, local hemodynamics, and temporal wall shear stress - should be considered. Therefore, in brief and without discussion of methods employed, several key concepts are mentioned. While the flow of blood is generally laminar, in some cases this flow can be disrupted and result in transitional conditions [Supplementary Figure 1], which may contribute to certain cardiovascular pathologies. Transitional flow in the presence of hypercholesterolemia has been proven to prime the vessel wall for the pathogenesis of atherosclerosis [61]. Transitional blood flow has also been suggested as a cause of post-stenotic dilatation, however this association may be due to the common occurrence of turbulence alongside stenosis of the blood vessel wall [62-63].

Transition has been proven to significantly increase pressure and shear stress within aneurysmal regions. Khanafer et al. [11,64] suggest that this may result in a self-perpetuating mechanism of further dilatation and subsequent increase of turbulence in the region. It has been suggested that the associated hemodynamics through an aneurysm, such as recirculating flow, may result in the formation of thrombosis [64,65]. The majority of the literature; however, supports the hypothesis that the formation of an aneurysm is a multi-factorial, degenerative process [66], not solely affected by hemodynamics and mechanical wall stress, but including inflammation and immune response, molecular genetics, and degradation of surrounding connective tissue [64].

Viscoelastic modelling

Large arteries are viscoelastic, which entails distinct mechanical behaviour compared to typical elastic models and calls for analysis of time-dependent behaviour. Due to this viscous component, there is energy retained within the arterial wall upon unloading, which is seen through hysteresis present in the stress-strain, and pressure-diameter curves of arteries [67,68]. Hysteresis loops may be used to estimate damping capacity, which is associated with the ratio of the dissipated energy to the stored energy [69]. An interesting study on strain-rate effect of mechanical properties by Delgadillo et al. [70] revealed that at a stretch ratio of 1.5, the experienced load within the arterial wall is reduced by 20% when the strain rate is increased from 10 to 200 %/S. They suggested that: “this behavior might be a consequence of the faster fluidization and small re-solidification that occurs in the cell at higher deformation rates”.

Torsion

While the response of arteries to axial stretching and circumferential stretching has long been studied and used to quantify failure of the arterial wall, the effect of torsion has been explored to a lesser extent, despite the fact that in vivo, arteries are often subject to twisting along the longitudinal direction with body movement [71,72]. Klein et al. [72] found that there was a significant change in arterial length, curvature and twist in the femoropopliteal arteries when subjects were cross legged compared to straight legged. Furthermore, the abdominal aorta and common iliac arteries exhibit significant morphological deformations from musculoskeletal motion. Hence, torsion is of particular concern since it has been identified as a possible contribution to failure of stents in the more mobile arteries [71-74]. Further study on the shortening, twisting and bending patterns of these arteries with stenting is required.

CONCLUSION

The inter-individual heterogeneity of the aorta’s geometry and composition, and the distinct differences in regional mechanical properties [66], fuel the difficulty behind understanding the underlying mechanics of the aorta. Uniaxial tests provide data regarding local mechanical properties and provide base comparisons between diseased and healthy arteries [4,8-10,12,75]. However, biaxial tests provide a better estimate of the multiaxial and anisotropic properties of arteries [14-18]. Both tests allow for data collection of incremental
elastic modulus until specimen failure, thus providing rudimentary data about rupture conditions. Bulge inflation tests further provide information on biaxial behaviour\textsuperscript{[5,22-24]}; however, inflation-extensions tests replicate \textit{in vivo} loading scenarios and are more suited to explore the in-depth loading mechanisms under which healthy tissue may become diseased\textsuperscript{[26-29]}. The opening angle test illustrates the residual stresses that are present in the circumferential direction in arterial walls\textsuperscript{[28,29,37,39]}. Lastly, the nanoindentation test may be used to characterize local deformation and inter-layer properties of arteries\textsuperscript{[10,41]}. There has been great effort put into quantification of the mechanical properties of the aorta; however, acquiring whole tissue is difficult and the importance of standard testing cannot be overlooked. The development of more robust \textit{in vitro} test methods and further research with respect to spatial and temporal variations in aortic composition will lead to a clearer understanding of the biomechanical contributions to vascular pathologies such as aneurysms. This will potentially lead to a more comprehensive stratification of aneurysm rupture risk and will hopefully lead to more targeted treatment strategies.

**DECLARATIONS**

**Authors’ contributions**

Performed literature review and composed manuscript: Pejcic S, Hassan SMA
Edited Manuscript: Rival DE, Bisleri G

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All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

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**Consent for publication**

Not applicable.

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