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In vitro characterisation of arterial stiffening: From the macro- to the nano-scale

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Abstract Accurate measurement of the material properties of arterial tissue is important for better characterisation of diseases and the development of reliable computational models. There are a number of in vitro techniques that are applied to study the biomechanical properties of arterial tissue. This review article presents data obtained using tensile testing, nanoindentation, scanning acoustic microscopy (SAM) and atomic force microscopy (AFM). Each of these techniques provides material property information at a different spatial resolution and in many ways are complementary techniques. The lack of consensus in the literature with regard to the appropriate stress and strain definitions that should be used when reporting tensile testing data is also highlighted. The potential of higher spatial resolution techniques, which provide data at micro-scale (nanoindentation and SAM) and nano-scale (AFM) for application to the characterisation of human aortic tissue are discussed. Finally, studies, which have examined age-related changed in the aorta at these different length scales, are highlighted.
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Introduction: structural basis of load transfer in arteries

Conduit arteries such as the aorta exhibit non-linear mechanical behaviour; the stiffness of arteries increases with distention. This non-linear behaviour is a protective feature preventing elastic instability such as that associated with aortic aneurysms.[1] The non-linear behaviour is derived from the composite structure of the vessel wall, where elastomeric elastin acts as the compliant phase and the relatively inextensible collagen as the stiffer, reinforcing phase. It is this composite structure of the vessel wall that provides the non-linear behaviour with the initial stiffness represented by the elasticity of elastin, whereas the higher stiffness at high strains represented by fully tensed collagen fibres.[1,2]

The structural basis of the load transfer from elastin to collagen is the medial lamellar unit (MLU) composed of

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concentric elastic lamellae with smooth muscle cells and collagen fibres within the inter-lamellar spacing.[3,4] As pressure increases, there is a progressive straightening of the lamellae with a corresponding decrease in the inter-lamellar distances.[1] This circumferential straightening of elastin layers and the alignment of collagen fibres with distention under physiological pressures correlates with the increasing elastic modulus observed by mechanical testing in vitro.[1]

Experimental observations with electron microscopy have led to the refinement of the original MLU model.[5,6] It is now clear that the MLU is highly complex and that the arrangement of the main constituents correlates with the known mechanical behaviour of the aorta. The three primary medial constituents (collagen bundles, smooth muscle cell nuclei and inter-lamellar elastin fibres) are all found to be predominantly orientated in the circumferential direction corresponding with the circumferentially dominant values of physiological stress.[6]

Hence, given that arteries have a multi-layered anatomical structure and a complicated organisation of cellular and extracellular matrix components within these layers, it would be expected that with ageing and vascular diseases associated with increased arterial stiffening, that there is mechanical degradation within arteries at the macroscopic level (encompassing the adventitia, media and intima), microscopic (within a specific anatomical layer or structural unit such as the MLU) or at the molecular or cellular level.[7] Similar to findings for cartilage,[8] it may be that decline in arterial structure begins at the molecular level and progresses to the higher levels of architecture. Hence, there is a need to determine localised mechanical properties for vascular tissue and relate these properties to its macroscopic structure and function. This will enable better characterisation of changes that occur due to the vasculature with ageing and disease, the development of reliable numerical models, and ultimately improved clinical treatments.

This review article will focus on a number of in vitro techniques that can be used determine the biomechanical properties of arteries at different length scales (macro- to nano-scale), and highlight data reported in the literature which considers age-related changes in arterial stiffening across these length scales.

Macromechanical testing

Uniaxial tensile tests where rectangular specimens are cut from vascular tissue, mechanically clamped and subjected to an axial tensile force (Fig. 1) yield a similar shaped stress–strain curve as an incremental elastic modulus-wall stress curve (which are widely used to characterise in vivo biomechanical properties of the arteries) e.g.[9,10] Tensile testing is a useful technique for biomechanical characterization of vascular tissues as a number of parameters can be extracted from the resulting stress–-strain curves.[11]

The mechanical properties of aortic aneurysm as compared to non-aneurysmal aorta have been determined in a number of studies using uniaxial tensile testing e.g.[13,14] The tensile strength of ascending thoracic aortic aneurysm tissue is found to be 29% and 34% less than that of control

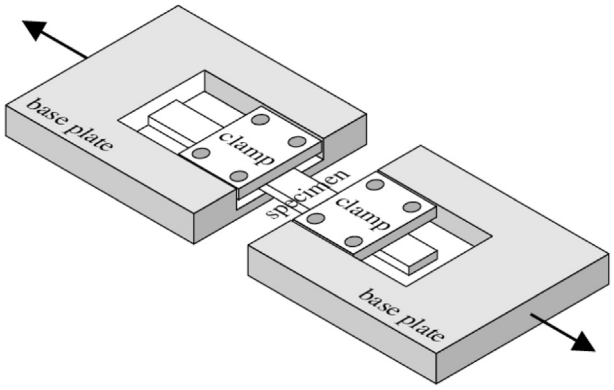

Figure 1 Schematic showing sample setup for an uniaxial tensile test. A rectangular section of tissue is prepared and clamped as shown. Reproduced from Ref. 12 with permission from Highwire Press.

tissue in the longitudinal and circumferential orientations respectively.[14]

One of the issues with the stress–strain behaviour reported in the literature for aortic aneurysm tissue using tensile testing is the definition of stress and strain.[11,15] The most commonly used definitions for stress and strain are engineering stress and engineering strain.[16] Here, it is assumed that stress (σ_E) is uniformly distributed over the cross-sectional area of the sample and is equal in magnitude to

$$\sigma_E = \frac{P}{A_o} \tag{1}$$

where P is the applied load and A_o is the original cross-sectional area.

Similarly, engineering strain (ε_T) is assumed to be constant over the sample gauge length (l) and equal to

$$\varepsilon_T = \frac{\Delta l}{l} \tag{2}$$

Although these definitions may be true in some engineering applications where the cross-sectional area and length of the specimen do not change substantially during loading, the relationships do not hold true for biological tissues where the cross-sectional area and the length of the specimen change substantially during testing. In such cases it is more appropriate to use alternative measures of stress and strain. True stress (σ_T) is defined as the ratio of the applied load to the instantaneous cross-sectional area (A) of the specimen. True stress can be related to the engineering stress by assuming there is no change in volume:

$$A \cdot l = A_o \cdot l_o \tag{3}$$

where l_o is the original specimen length.

Hence,

$$\sigma_T = \frac{P}{A} = \frac{P}{A_o} \cdot \frac{l}{l_o} = \sigma_E(1 + \varepsilon_E) \tag{4}$$

True strain is defined as the sum of all the instantaneous engineering strains.

$$\mathrm{d}\varepsilon = \frac{\Delta l}{l} \tag{5}$$

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