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Research Paper

Frequency-modulated atomic force microscopy localises viscoelastic remodelling in the ageing sheep aorta

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ABSTRACT

Age-related aortic stiffening is associated with cardiovascular diseases such as heart failure. The mechanical functions of the main structural components of the aorta, such as collagen and elastin, are determined in part by their organisation at the micrometer length scale. With age and disease both components undergo aberrant remodelling, hence, there is a need for accurate characterisation of the biomechanical properties at this length scale. In this study we used a frequency-modulated atomic force microscopy (FM-AFM) technique on a model of ageing in female sheep aorta (young: ~18 months, old: >8 years) to measure the micromechanical properties of the medial layer of the ascending aorta. The novelty of our FM-AFM method, operated at 30 kHz, is that it is non-contact and can be performed on a conventional AFM using the 'cantilever tune' mode, with a spatial (areal) resolution of around 1.6 μm^2. We found significant changes in the elastic and viscoelastic properties within the medial lamellar unit (elastic lamellae and adjacent inter-lamellar space) with age. In particular, there was an increase in elastic modulus (Young; geometric mean (geometric SD)=42.9 (2.26) kPa, Old=113.9 (2.57) kPa, $P<0.0001$), G' and G'' (storage and loss modulus respectively) (Young; $G'=14.3$ (2.26) kPa, Old $G'=38.0$ (2.57) kPa, $P<0.0001$; Young; $G''=14.5$ (2.56) kPa, Old $G''=32.8$ (2.52) kPa, $P<0.0001$). The trends observed in the elastic properties with FM-AFM matched those we have previously found using scanning acoustic microscopy (SAM). The utility of the FM-AFM method is that it does not require custom AFM hardware and can be used to simultaneously determine the elastic and viscoelastic behaviour of a biological sample.

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1. Introduction

Ageing is associated with arterial stiffening and the development of cardiovascular disease. Stiffening in large arteries such as the aorta is typically studied at the level of the whole vessel. However, there is now evidence which suggests that alterations in the structure and mechanical properties of large arteries at the micron scale can have detrimental effects on the function of the aorta (Kohn et al., 2015). Hence, there is growing interest in characterizing the nano- and micro-mechanical properties of the aorta to better understand the structure-property-function relationships within large arteries (Akhtar et al., 2014, 2009; Grant and Twigg, 2013; Kohn et al., 2015). Due to the intricate organisation of extracellular matrix (ECM) components within the vessel wall, there is a need to develop methods that accurately measure the mechanical properties of the tissue with high spatial resolution. However, due to a lack of appropriate techniques, there is still limited information on these properties at the micron length scale (Graham et al., 2011). Accessing this information is vital because both force-sensing cells and large ECM assemblies such as collagen and elastic fibres, which govern the mechanical response of large arteries, are organised at this length scale (Akhtar et al., 2011). Hence, there is a need to develop reliable, quantitative methods for the assessment of the mechanical properties of vascular tissue at the micron length scale (Akhtar, 2014; Akhtar et al., 2009).

Our previous work focussed on the use of scanning acoustic microscopy (SAM) to determine regional variations in the elastic properties of the aorta at this length scale (Akhtar et al., 2014; Graham et al., 2011; Lopez-Andres et al., 2012). SAM measures the speed of longitudinal acoustic waves through a material, which can be related to its elastic modulus. When SAM is operated at frequencies close to 1 GHz, it provides quantitative measurements of acoustic wave speed with a linear spatial resolution around 1 μm. Using this technique we have shown that changes in the micromechanical properties of the aorta that occur with age and with pathology (diabetes), can be localised to the inter-lamellar regions of the medial lamellar unit (MLU) within the aorta. The MLU is the main load-bearing component of the aortic wall and is composed of an elastin-rich lamellae and a fibrillar collagen with a vascular smooth muscle cell-rich inter-lamellar region (O'Connell et al., 2008).

Although the use of SAM for measuring the properties of soft tissues such as the aorta has a number of advantages, including co-localisation of histological and elastic properties and fast data acquisition (Zhao et al., 2012), SAM cannot provide information on the viscoelastic and time-dependant response of tissue, because it operates at low amplitude acoustic deformation rates, and is limited to characterising tissue stiffness indirectly because acoustic wave speed is a function of both elastic modulus and local density. Another technique for acquiring mechanical properties is a contact or indentation method such as atomic force microscopy (AFM) which can be used to measure the elastic and viscoelastic properties of tissues at a higher spatial resolution than SAM, and has been used, for example, to characterise intimal stiffening in the aorta (Huynh et al., 2011).

Despite the relatively wide use of AFM for biological applications, AFM-indentation approaches are not always ideal for probing soft biological tissues due to lengthy acquisition times and also the requirement of the probe to be either continuously or intermittently in contact with the sample. A comparison of acoustic and AFM methods for the characterisation of tissue has been reviewed elsewhere (Akhtar et al., 2011).

Frequency-modulated atomic force microscopy (FM-AFM) is an AFM mode which overcomes some of these issues and can be employed by existing commercial AFM systems. The principle of FM-AFM is that it relies on detecting small changes in the cantilever resonant frequency, which occur in response to the tip–sample interaction (Higgins et al., 2005). These frequency shifts can then be used to measure force interactions between the tip and sample. Although there are concerns about uncertainty when AFM techniques are used for mechanical property characterisation e.g. due to complications arising due to tip-sample interactions (Lin et al., 2009), AFM methods are nevertheless powerful tools for the nano- and micro-scale characterisation of soft tissues. When probing the difference between two samples, any uncertainty can be minimised by utilising the same tip and configuration to provide more robust mechanical property data.

FM-AFM is a powerful tool for studying the mechanical properties of single biomolecular interactions and force-extension response of isolated molecules in liquid (Higgins et al., 2005; Humphris et al., 2000). FM-AFM has previously been applied to biological systems to study single polysaccharide molecules (Humphris et al., 2000) and DNA (Cerreta et al., 2013), for example. Gavara and Chadwick (2010) developed the technique further, specifically for applications in the study of the microrheology of biological tissues that produce or detect sound. In that work, they demonstrated that material properties of gels

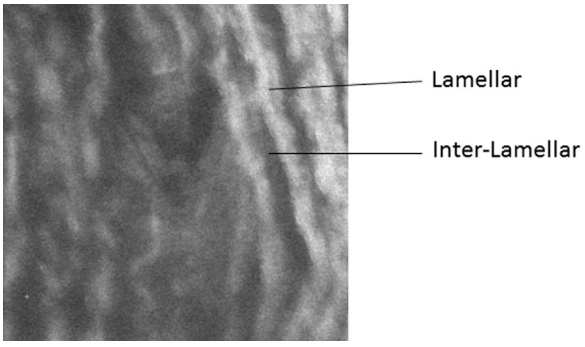

Fig. 1 – Example optical image captured with the integrated microscope for localisation of lamellar and inter-lamellar regions of the medial layer. The size of the image 150 × 150 μm obtained with a 40 × objective (880 × 880 pixels).

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