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Research paper

Anisotropic time-dependant behaviour of the aortic valve

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ABSTRACT

The complex tri-layered structure of the aortic valve (AV) results in anisotropic quasi–static mechanical behaviour. However, its influence on AV viscoelasticity remains poorly understood. Viscoelasticity may strongly influence AV dynamic mechanical behaviour, making it essential to characterise the time-dependent response for designing successful substitutes. This study attempts to characterise the time-dependent behaviour of the AV at different strain and load increments, and to gain insight into the contribution of the microstructure to this behaviour. Uniaxial incremental stress-relaxation and creep experiments were undertaken, and the experimental data analysed with a generalised Maxwell model, to determine the characteristic time-dependent parameters. Results showed that the time dependent response of the tissue differed with the loading direction, and also with the level of applied load or strain, in both stress-relaxation and creep phenomena. Both phenomena were consistently more pronounced in the radial loading direction. Fitting of the Maxwell model highlighted that the time dependent modes required to model the data also varied in different increments, and additionally with the loading direction. These results suggest that different micro-structural mechanisms may be activated in stress-relaxation and creep, determined by the microstructural organisation of the valve matrix in each loading direction, at each strain or load increment.

1. Introduction

The material properties of the native aortic valve (AV) have typically been investigated using quasi-static tensile tests, revealing that the tissue is highly anisotropic (Billiar and Sacks, 2000; Missirlis and Chong, 1978; Sauren et al., 1983; Stella and Sacks, 2007). However, the AV also exhibits time-dependent behaviour when subjected to specific loading conditions similar to other collagenous connective tissues, such as tendons and ligaments (Doehring et al., 2004; Fung, 1993; Pioletti and Rakotomanaana, 2000). Such time-dependent viscoelastic behaviour can be realised through either stress-relaxation or creep tests. The former reflects a decrease in load (stress) which occurs when the tissues are subjected to a constant elongation (strain), while creep describes the increasing elongation (strain) of the tissue under constant load (stress) (Stella et al., 2007; Thornton et al., 1997). These processes will also occur when samples are subjected to continuous cyclic perturbations, under either load or strain control (Thornton et al., 1997).

The native AV functions under repeated cyclic loading *in vivo*, experiencing typical stresses in the range of 250–400 kPa, over an average of 3×10^9 cycles in a lifetime (Merryman et al., 2009; Stella and Sacks, 2007; Yacoub and Cohn, 2004).

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Under such repetitive loading, time-dependent phenomena can strongly influence the dynamic mechanical behaviour of any material, most notably its fatigue life characteristics (Grell et al., 2007; Rao and Farris, 2008; Wang and Ker, 1995; Wren et al., 2003). While the healthy native AV is highly fatigue resistant, mismatch of the viscoelastic response of prosthetic valves compared with native tissue can significantly limit their functional lifetime (Lim and Boughner, 1976; Wells and Sacks, 2002). Characterising viscoelastic behaviour of the valve is thus of critical importance in predicting the dynamic life of natural valves and providing design features for efficient replacements.

A recent approach to characterising mechanics of the AV involves the use of *in vitro* biaxial loading regimes, as a means of providing more physiologically representative loading environment experienced by native valve (Billiar and Sacks, 2000; Stella and Sacks, 2007). However, of the few studies examining the time-dependant behaviour, the results have indicated that heart valve tissues may not exhibit a complete range of time-dependant phenomena under biaxial loading mode, as no measurable creep has been reported in those studies (Liao et al., 2007; Stella et al., 2007). The observed lack of biaxial creep behaviour has indeed been attributed to the complex effects of orthogonal loading on fibre kinematics under biaxial loading regime (Liao et al., 2007; Stella et al., 2007). Hence, while a biaxial loading protocol is more physiologically relevant, the resulting fibre kinematics conditions inhibit characterisation of AV creep behaviour.

Time-dependent spectra cannot be measured directly in experiments (Baumgaertel and Winter, 1992) and thus experimental data are generally fitted to viscoelastic models, to estimate the associated moduli and characteristic times (Baumgaertel and Winter, 1992; Doehring et al., 2004; Stella et al., 2007). This involves the adaptation of appropriate theoretical criteria and employment of suitable viscoelastic models for a proper interpretation of data and characterisation of time-dependent properties in tissues. While the quasi-linear viscoelasticity (QLV) incorporates a well-established theoretical criterion in describing the time-dependant behaviour of soft tissues, studies have indicated that the stress-relaxation of AV cannot be fully described by the QLV model (Sauren et al., 1983; Sauren and Rousseau, 1983; Stella et al., 2007). Indeed, QLV assumes that stress relaxation is independent of applied strain (Provenzano et al., 2002), an assumption which has not been confirmed in the relevant experiments.

Adaptation of the uniaxial loading mode and alternative modelling criteria would thus provide more insights in assessment of the time-dependant behaviour of AV and identification of the structural basis of the mechanisms facilitating such behaviour. Accordingly, in this study, AV leaflets were subjected to uniaxial stress-relaxation and creep tests in both circumferential and radial directions, in order to investigate the anisotropy, and gain new insights into the role of matrix components, in the time-dependant behaviour. Different strain and load increments were employed to assess the dependence of stress-relaxation and creep parameters, respectively, on the level of the applied stimuli. The experimental data were then modelled with a generalised Maxwell model to establish how the AV microstructure contributes to the resulting observed phenomena in each of the test conditions.

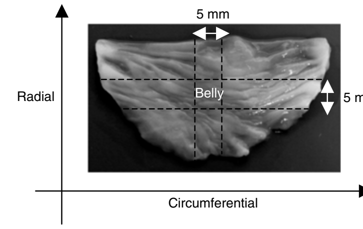

Fig. 1 – Image of an AV leaflet from which 5 mm wide strips were cut from the belly region, in either the circumferential or radial direction.

2. Materials and methods

22 porcine hearts were obtained from animals between 18 and 24 months, from a local abattoir within two hours of slaughter. The three AV leaflets were dissected from the aortic root and maintained in Dulbecco's Modified Eagle's Medium (DMEM, Sigma, Poole, UK), at 25 °C. From each leaflet a 5 mm wide circumferential or radial strip, was excised from the central (belly) region, as shown in Fig. 1. A total 33 pairs of samples were included in the tests.

The thickness of each sample was measured, using a non-contact laser micrometer (LSM-501, Mitotuyo, Japan; resolution = 0.5 μm). Each sample was moved through the laser beam, and thickness measurements recorded at 1 mm intervals. The mean thickness was then used to determine the cross-sectional area of each sample. The samples were returned to DMEM before performing the experiments. All experimental tests were carried out within four hours of excision of the tissue strips.

The mechanical tests were performed using a material testing machine (Bionix 100, MTS, Cirencester, UK), fitted with custom designed pneumatic grips each with a corrugated surface, to securely grip the samples and prevent slippage during the experiments. The distance between the grips was set at 10 mm for all test protocols. Prior to the start of each test, a tare load of 0.01 N was applied to the specimens, to establish a consistent zero position. The force–elongation data was recorded at a frequency of 20 Hz. During the tests, hydration of the samples was maintained by spraying with DMEM.

Preliminary quasi-static tests to failure were carried out on 15 pairs of samples, at a strain rate of 60%/min, in order to determine the mean load and deformation at failure. These data were then used to identify the range of load or strain increments for stress-relaxation and creep tests, given as percentages of the ultimate failure parameters.

2.1. *Incremental stress-relaxation protocol*

9 pairs of samples were subjected to incremental tensile stress-relaxation tests. Samples were strained to eight different increments of strain, between 3% and 90% of the