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Original article

**Viscoelastic properties of mitral valve leaflets: an analysis of regional variation and frequency-dependency.**

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## **ABSTRACT**

The aim of this study was to determine the regional variation in viscoelastic properties of mitral valve leaflets over a range of physiological and patho-physiological frequencies. This included comparisons to be made between anterior and posterior leaflets, anterior leaflet clear and rough zones, and radial and circumferential leaflet orientation. Dynamic Mechanical Analysis (DMA) was used to determine frequency-dependent viscoelastic properties. The valve leaflets were dissected from eight porcine hearts. The leaflets were loaded under a sinusoidal tensile displacement, with a mean dynamic peak to trough strain of 11%, applied to all leaflet samples at 9 different frequencies, ranging from 0.5 to 10 Hz. The anterior leaflet has higher storage and loss stiffness than the posterior leaflet. The storage stiffness of circumferential tissue is greater than that of radially orientated valve tissue ( $2.0 \pm 1.6$  N/mm cf.  $1.7 \pm 0.9$  N/mm;  $p < 0.05$ ); however, the loss stiffness is greater for radial tissue ( $0.15 \pm 0.07$  cf.  $0.14 \pm 0.09$  N/mm;  $p < 0.05$ ). Likewise, the storage stiffness of the anterior leaflet clear zone is greater than that of the rough zone ( $2.4 \pm 1.6$  cf.  $2.1 \pm 1.2$ ;  $p < 0.05$ ), but the loss stiffness is greater for the rough zone ( $0.17 \pm 0.09$  N/mm cf.  $0.14 \pm 0.08$  N/mm;  $p < 0.05$ ). In conclusion, the viscoelastic properties of porcine mitral valve leaflets have regional variations, with dynamic stiffness being dependent on circumferential or radial orientation and on location at a clear or rough zones.

**Keywords:** Heart, Loss, Mechanical properties, Mitral valve, Stiffness, Storage, Viscoelasticity.

## Introduction

The mitral valve is found between the left atrium and left ventricle, and allows forward flow but prevents backflow of blood through the valve when closed. Its two leaflets, anterior and posterior, are connected to the papillary muscles on the left ventricular muscle wall via chordae tendineae.<sup>1,2</sup> Failure of the mitral valve can result in either stenosis during diastole or regurgitation during systole, which can require surgical repair or replacement.<sup>3,4</sup>

Measuring the mechanical properties of mitral valve leaflets allows for requirements of replacement materials to be characterised.<sup>5</sup> A range of mechanical properties have been determined for mitral valve leaflets, including at different strain rates, surface strains, stress relaxation and creep.<sup>6, 7,8</sup> However, the dynamic mechanical properties of mitral leaflets have undergone limited analysis.

Dynamic Mechanical Analysis (DMA) can be used to apply a sinusoidal tensile strain with the resultant tensile force subsequently measured.<sup>9-13</sup> For viscoelastic materials the displacement is out of phase with the load by an angle  $\delta$ . Thus, the viscoelastic properties, including storage,  $E'$ , and loss,  $E''$ , moduli can be determined for a material.<sup>9,14</sup> Likewise, the storage,  $k'$ , and loss,  $k''$ , stiffness can be determined for a structure. The storage stiffness characterises the ability of the structure to undergo elastic recoil (energy stored) and the loss stiffness characterises the ability of the structure to dissipate energy.<sup>15</sup>

The frequency dependent viscoelastic properties of heart valve leaflets have been measured<sup>16,17</sup> between 0.5 to 5 Hz. However, such frequencies do not account for the maximum strain rates<sup>6,7</sup> to which leaflets may be exposed. Moreover, no distinction was made between mitral valve anterior leaflet clear and rough zones, and posterior leaflets, or their variation between radial and circumferential orientations.

The aim of this project was to determine whether there are regional variations in viscoelastic properties of mitral valve leaflets. DMA has been used to determine the dependency of storage and loss stiffness over frequencies relevant to physiological and patho-physiological heart rates. Viscoelastic properties have been compared across porcine anterior and posterior mitral valve leaflets, including variation with radial and circumferential leaflet orientation, and with rough and clear zones for anterior leaflets. Note, unlike the anterior leaflet, the posterior leaflet is not typically considered to have distinct rough and clear zones.

## **Methods**

### *Specimens*

Porcine hearts were obtained from a supplier (Fresh Tissue Supplies, East Sussex) and delivered frozen. Upon arrival at the laboratory they were stored in heat-sealed bags, soaked in Ringer's solution, and stored at  $-40^{\circ}\text{C}$ , similar to previous protocols used for mitral heart valves.<sup>9,18,19</sup> On the day of testing hearts were thawed, ready for dissection and testing of valve leaflets.<sup>20</sup> Mitral valve leaflets were dissected from eight porcine hearts, obtaining anterior leaflet clear zone, anterior leaflet rough zone, and posterior leaflet samples. Square samples were cut with sides parallel to the radial and circumferential orientation (Figure 1). The leaflets were cut into square specimens with dimensions of  $10\text{ mm} \times 10\text{ mm}$ .

### *Mechanical testing and viscoelastic characterisation*

A material testing machine (ELF 3200, Bose Corporation, ElectroForce Systems Group, Minnesota, MN, USA), operated using WinTest Dynamic Mechanical Analysis software was used for all tests. Viscoelastic characterisation involved the following. Fourier analysis of the measured force and out-of-phase displacement waves was performed. From the Fourier analysis, the magnitude of the force, the magnitude of the displacement, phase lag ( $\delta$ ) between force and displacement waves and the frequency of these waves were determined. Subsequently the magnitude of the complex stiffness,  $k^*$ ,

was calculated (i.e. the ratio of the magnitude of the force to the magnitude of the displacement). Storage,  $k'$ , and loss,  $k''$ , stiffness were then calculated using equations 1 and 2, respectively.<sup>9,10,11</sup>

$$k' = k \cdot \cos(\delta) \quad 1$$

$$k'' = k \cdot \sin(\delta) \quad 2$$

For mechanical testing, samples were placed between two clamps with sandpaper to prevent slipping, described further elsewhere.<sup>19</sup> The specimens were unloaded when gripped; the slack was then removed and samples were stretched to a test dimension of 10 mm which introduced a pre-strain of 11%. The specimens were maintained hydrated throughout testing by loosely wrapping tissue paper saturated in Ringer's solution.<sup>9,21</sup>

DMA was performed by sinusoidally loading samples under displacement control, between 1.5 mm and 2.5 mm displacement. Samples were loaded at nine frequencies between 0.5 - 10 Hz. This range of frequencies was chosen to cover bradycardia heart rates (30 bpm or 0.5 Hz), physiological heart rates (60-70 bpm or 1-1.2 Hz), exercise heart rates (180 bpm or 3 Hz) and heart rates corresponding to tachycardia (300 bpm or 5 Hz).<sup>22</sup> Higher frequencies (5 - 10 Hz) were included to characterise samples up to frequencies equivalent to their maximum estimated strain rates of approximately between 500 to 1000 % $\cdot$ s<sup>-1</sup>.<sup>6,7</sup> Preconditioning loading cycles were applied at 1 Hz for 200 cycles at 1 Hz. This is the equivalent to physiological heart

rate for over 3 minutes and ensures repeatability between samples. The order of testing of sample orientation was varied to circumvent any misleading trends which might ensue from repeat testing of samples. Thus, half the samples were initially tested radially and the other half circumferentially. No differences were identified in the data obtained between samples tested first and second per given orientation (i.e. radial or circumferential).

#### *Data analysis*

An Anderson-Darling normality test was used to assess whether data had a normal distribution; however, the obtained data was not normally distributed ( $p < 0.05$ ). Thus, reference is made to median, rather than mean, results. Further, statistical comparisons were assessed using a Wilcoxon Signed Rank test ( $p < 0.05$ ). This is a non-parametric test for paired data.<sup>23</sup> All statistical analysis was performed using Minitab (Minitab 16, Minitab Ltd, Coventry, UK).



## Results

### *Storage stiffness*

The storage stiffness,  $k'$ , was found to be frequency-dependent.  $k'$  followed a second-order polynomial trend with frequency,  $f$ , in the form of equation 3. However, the result was that between 0.5 to 5 Hz,  $k'$  remained approximately constant for each individual sample; then, decreasing slightly between 6 and 10 Hz (Figure 2).

$$k' = Af^2 + Bf + C \quad 3$$

Here,  $A$ ,  $B$  and  $C$  are constants.

At 1 Hz,  $k'$  ranged from  $2.2 \pm 1.3$  N/mm for anterior leaflet rough circumferential samples, to  $1.6 \pm 0.3$  N/mm for posterior leaflet radial samples (Table 1). Anterior leaflets had higher values of  $k'$  than posterior leaflets.

$k'$  was significantly greater along the circumferential as compared to the radial orientation, as assessed through paired comparisons for anterior leaflet clear and rough zones, and posterior leaflets. The circumferential and radial median  $k'$  values were  $2.0 \pm 1.6$  N/mm and  $1.7 \pm 0.9$  N/mm, respectively ( $p < 0.05$ ; Table 2). Across the full frequency-sweep assessed, the anterior leaflet,  $k'$  was significantly greater for specimens from the clear as compared to rough zone, with median values of  $2.4 \pm 1.6$  N/mm and  $2.1 \pm 1.2$  N/mm, respectively ( $p < 0.05$ ; Table 3).

### *Loss stiffness*

The loss stiffness,  $k''$ , was frequency-independent (Figure 2), remaining constant between frequencies from 0.5 up to 10 Hz. It was approximately an order of magnitude lower than  $k'$ . At 1 Hz, median values for  $k''$  ranged from  $0.19 \pm 0.06$  N/mm for anterior leaflet clear circumferential samples, to 0.14 N/mm for anterior leaflet clear radial ( $\pm 0.03$  N/mm), posterior leaflet circumferential ( $\pm 0.04$  N/mm) and radial ( $\pm 0.03$  N/mm) samples (Table 4). Anterior leaflets had higher values of  $k''$  than posterior leaflets.

$k''$  was significantly greater along the radial as compared to circumferential orientation, as assessed through paired comparisons for anterior leaflet clear and rough zones, and posterior leaflets. The radial and circumferential median  $k''$  values were  $0.15 \pm 0.07$  N/mm and  $0.14 \pm 0.09$  N/mm, respectively ( $p < 0.05$ ; Table 2). For the anterior leaflet,  $k''$  was significantly greater for specimens from the rough as compared to the clear zone, with median values of  $0.17 \pm 0.09$  N/mm and  $0.14 \pm 0.08$  N/mm, respectively ( $p < 0.05$ ; Table 3).

## Discussion

The viscoelastic properties of the mitral valve leaflets have been measured using DMA. Storage and loss stiffness were compared for the anterior and posterior leaflets, including assessment of radial and circumferential orientation. In this study, it has been found that:

- storage and loss stiffness were greater for the anterior leaflet than for the posterior leaflet;
- storage stiffness was greater circumferentially than radially;
- storage stiffness was greater for anterior leaflet clear zone than for the rough zone;
- loss stiffness was greater radially than circumferentially;
- loss stiffness was greater for anterior leaflet rough zone than for the clear zone.

The storage stiffness being greater than the loss stiffness is consistent with other heart valve tissues.<sup>9,16,17</sup> Previously, storage and loss moduli had been reported, of around 26 N/mm<sup>2</sup> and 1 N/mm<sup>2</sup> respectively.<sup>16</sup> Although the storage to loss ratio is consistent with our study, the values are approximately an order of magnitude above those measured in this present study. This may be due to differences in strain used in the two studies, and differences in testing human versus porcine mitral valve leaflets. Although, it has previously been suggested that smaller strain may return a larger storage modulus for certain materials,<sup>24</sup> consistent with the higher strain used in our study, given the non-linearity of connective tissues (increased stress with strain)

differences may more likely reflect differences between porcine and human tissues. In our study, we have used a with a mean dynamic peak to trough strain of 11%, with a mean of 33% strain, comparable to peak strains of 22% which has been measured *in vivo*.<sup>25</sup>

This present study found the storage stiffness to be frequency-dependent, with the loss stiffness frequency-independent. This is consistent with viscoelastic properties of mitral valve chordae tendineae.<sup>9</sup> However, the frequency-dependency of the storage stiffness in this present study was minimal when compared to tissues such as articular cartilage,<sup>10</sup> bladder tissue<sup>26</sup> and tumours.<sup>27</sup> Articular cartilage, for example, undergoes a glass transition which is identifiable with a frequency sweep,<sup>10,13</sup> presumably reflecting its much higher water content. When compared to mitral valve chordae tendineae,<sup>9</sup> mitral valve leaflets also have a much lower storage and loss stiffness. This is likely the result of chordae tendineae having a much higher collagen content and greater alignment with the axial loading direction than mitral valve leaflets.

Collagen is aligned circumferentially in mitral valve leaflets.<sup>28</sup> This is consistent with our finding of lower storage stiffness for mitral valve leaflets in the radial orientation when compared to a circumferential orientation. Which is, furthermore, consistent with heart valve leaflets being stiffer in circumferential orientations.<sup>29,30</sup> However, for loss stiffness the opposite was true, with radial tissue having higher values than circumferential values. This could relate to glycosaminoglycan distribution,<sup>31</sup> or

differences in the fibre-matrix interactions which lead to energy dissipation.<sup>32</sup> Further understanding as to how tissue components (e.g. collagen, glycosaminoglycans) influence the viscoelastic behaviour of natural tissues is of potential value to developing improved constructs for valve leaflet repair/replacement through tissue regeneration.

Mitral valve anterior leaflet chordae have previously been found to be stiffer than posterior leaflet chordae<sup>9,19</sup> which is consistent with the present finding for both storage and loss stiffness of mitral valve leaflets. This highlights the importance of comparing mechanical properties of distinct anterior leaflet regions of the mitral valve. Physiologically, chordae insert into the rough zone of the anterior leaflet but not into its clear zone.<sup>3</sup> This may reflect greater deflection of the rough zone of the mitral valve anterior leaflet during valve opening and closure, in which case mitral valve computational models<sup>33,34</sup> should aim to consider such mechanical behaviour. Further, viscoelastic characterisation of natural valve leaflets has potential applications for benchmarking novel replacement materials which may be under development.

### *Limitations*

In this study, stiffness has been used for comparisons rather than modulus. There are examples in literature where stiffness has been used for comparisons when using the self-same specimens, such as for hydration studies.<sup>10</sup> In our current study, all conclusions drawn can be supported through the use of stiffness, while having the

advantage of negating errors associated with measurements of dimensions; specifically, difficulties associated with accurately measuring leaflet thickness. This is because a modulus is calculated by normalising a stiffness (equations 4 and 5) using a given shape factor,  $S$  (equation 6) which is dependent on the samples thickness and surface area.<sup>19</sup>

$$E' = \frac{k'}{S} \quad 4$$

$$E'' = \frac{k''}{S} \quad 5$$

$$S = \frac{wt}{l} \quad 6$$

Here,  $w$  is width,  $t$  is thickness and  $l$  is length of the specimen sample.

An elastic modulus would be comparable to a complex modulus,  $E^*$ , of a viscoelastic material, where  $E^*$  is dependent on  $E'$  and  $E''$  (equation 7).

$$E^* = \sqrt{(E')^2 + (E'')^2} \quad 7$$

The caveat in comparing an elastic modulus and  $E^*$  is that as viscoelastic properties are rate dependent, rates of loading used for characterisation must be comparable. Using equations 4 to 7 to derive  $E^*$  leads to values mostly in the 1.0 – 2.5 MPa range for our study (outer limits of 0.43 to 5.61 MPa). This compares to our own measurements of elastic moduli for mitral valve leaflets of 2 - 5 MPa, which we have used previously in computational models of the mitral valve.<sup>33</sup>

The advantage of viscoelastic characterisation is that it includes components associated with storage and loss of energy, within the tissue, relevant to elastic recoil.<sup>14</sup>

The advantage of using dynamic mechanical analysis for such characterisation is that it enables the viscoelastic properties to be measured quantitatively; as has been performed in this current study on mitral valve leaflets. Although  $k'$  and  $k''$  did not vary much over the frequency range of 0.5 to 10 Hz, this should not be interpreted as meaning that characterising mitral valve leaflets at physiological frequencies (and/or loading rates) is not important. For other soft connective tissues, for instance, it has been found that the ratio of  $k':k''$  varies by an order of magnitude between physiological (e.g. 1 Hz) and below physiological (e.g. 0.1 Hz) loading frequencies.<sup>35</sup>

In this study, it was generally feasible to identify a 10 mm by 10 mm section of anterior leaflet rough zone within the porcine mitral valve specimens used. When stretched out, the rough zone of the anterior leaflet can be of comparable area to the clear zone.<sup>3</sup> However, it is feasible that for smaller valves, small sections of clear zone anterior leaflet may have been present within rough zone samples. Any such presence would have been minimal though, as during experimentation this was not typically observed. Further, any such presence would have led to minor under-predictions in differences in storage and loss stiffness between rough and clear zone samples.

There is some evidence of stiffening of tissues when comparing frozen to fresh human mitral leaflets,<sup>36</sup> however, the data included extensive overlap in results between fresh and frozen specimens. More recent studies have suggested that freezing temperature<sup>37</sup> and method of freezing preservation<sup>38</sup> may be more important. For this

current study, a protocol of storing tissues at -40 °C was followed,<sup>39</sup> consistent with recommended procedures to maintain initial stress-strain behaviour of soft connective tissues.<sup>40</sup>

## **Conclusion**

The viscoelastic properties of porcine mitral valve leaflets have regional variations. The anterior leaflet has higher storage and loss stiffness than the posterior leaflet. The storage stiffness of circumferential tissue is greater than that of radially orientated valve tissue; however, the loss stiffness is greater for radial tissue. Likewise, the storage stiffness of the anterior leaflet clear zone is greater than that of the rough zone, but the loss stiffness is greater for the rough zone.

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## FIGURE CAPTIONS

**Figure 1.** Mitral valve specimen. The anterior and posterior leaflets are shown. The square boxes show the samples dissected for testing.

**Figure 2.** Frequency-dependent viscoelastic properties of mitral valve leaflets. Data for four individual tissue samples is included. The storage stiffness of samples are shown using black markers, while loss stiffness of samples is shown using grey marker. Triangles denote tissue samples obtained from one mitral valve, squares denote tissue samples obtained from a second mitral valve. Dashed lines denote a circumferential test orientation, and full lines denote a radial test orientation.

## TABLES

**Table 1.** Regional storage stiffness for mitral valve leaflets when tested at 1 Hz. Note, SD: Standard Deviation.

leaflet	type	orientation	n	mean $k'$ (N/mm)	SD	median $k'$ (N/mm)
anterior	rough	circumferential	8	2.1	1.3	2.2
anterior	rough	radial	6	2.5	1.4	2.1
anterior	clear	circumferential	8	2.3	2.1	1.9
anterior	clear	radial	6	1.9	0.4	1.9
posterior	n/a	circumferential	8	1.5	0.7	1.7
posterior	n/a	radial	6	1.6	0.3	1.6

**Table 2.** Radial and circumferential viscoelastic properties of mitral valve leaflets.

Note, SD: Standard Deviation; \*denotes significant difference ( $p < 0.05$ ) between radial and circumferential.

	mean $k'$ (N/mm)	median $k'$ (N/mm)	SD	mean $k''$ (N/mm)	median $k''$ (N/mm)	SD
Radial	1.9*	1.7	0.9	0.17*	0.15	0.07
Circumferential	2.3	2.0	1.6	0.15	0.14	0.09



**Table 3.** Anterior leaflet rough and clear zone viscoelastic properties. Note, SD:

Standard Deviation; \*denotes significant difference ( $p < 0.05$ ) between clear and rough.

	mean $k'$	median $k'$	SD	mean $k''$	median $k''$	SD
Clear	2.7*	2.4	1.6	0.15*	0.14	0.08
Rough	2.3	2.1	1.2	0.19	0.17	0.09

**Table 4.** Regional loss stiffness for mitral valve leaflets when tested at 1 Hz. Note, SD:

Standard Deviation.

leaflet	type	orientation	n	mean $k''$ (N/mm)	SD	median $k''$ (N/mm)
anterior	rough	circumferential	7	0.18	0.08	0.17
anterior	rough	radial	6	0.21	0.11	0.18
anterior	clear	circumferential	5	0.16	0.06	0.19
anterior	clear	radial	6	0.14	0.03	0.14
posterior	n/a	circumferential	7	0.14	0.04	0.14
posterior	n/a	radial	6	0.14	0.03	0.14