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Frequency Dependent Viscoelastic Properties of Porcine Bladder

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Abstract

The aim of this study was to measure the viscoelastic properties of bladder tissue. Porcine bladders were dissected into rectangular strips and loops. Dynamic Mechanical Analysis was used to measure the viscoelastic properties of the bladder tissue (storage and loss stiffness) tested in a frequency range of up to 10 Hz. Storage stiffness was found to be consistently higher than loss stiffness. Average storage stiffness was found to be 1.89 N/mm and 0.74 N/mm for looped and rectangular samples, respectively. Average loss stiffness was found to be 0.24 N/mm and 0.11 N/mm for looped and rectangular samples, respectively. The results of this study are important for computational modelling of the bladder and for ensuring that tissue engineered bladder tissues have physiological viscoelastic properties.

Keywords

Bladder; Dynamic Mechanical Analysis; Loss; Mechanical Properties; Porcine; Stiffness; Storage; Viscoelasticity.

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1. Introduction

The human urinary bladder is an organ which stores urine. It has two inlets and one outlet in the form of the ureters and the urethra, respectively. The bladder usually holds around 400 ml of urine before giving a sensation of fullness (Guyton and Hall, 2011); however, this is dependent on many factors including the size of the person. The bladder wall is comprised of several layers; these are, from the luminal surface outwards: the mucosa (which includes the transitional epithelium), the submucosa (lamina propria), the detrusor muscle (muscularis propria), and the adventitia (Stevens and Lowe, 1997).

In the UK there are approximately 10,200 new cases and 5,000 deaths attributed to bladder cancer per year (Bladder cancer key facts, 2014), and for the USA and EU these figures have been estimated at 72,570/15,210 and 123,135/40,252 (Burger et al., 2013), respectively. At presentation over 75-85% will be non-muscle-invasive tumours (NMIBC, stages Ta/T1/Tis), with the remainder being muscle-invasive (MIBC, stages T2-4) (Lorusso *et al.,* 2005; van Rhijn *et al.,* 2009; Wallace *et al.,* 2002; Kaufman *et al.,* 2009). Many of those affected with MIBC undergo surgery to remove the bladder, radical cystectomy (Kaufman *et al.,* 2009; Witjes *et al.,* 2013), and in some of these cases the bladder is replaced with a substitute, such as part of the small or large intestine (Witjes *et al.,* 2013). However, patients undergoing bladder substitution need to be carefully selected (Nagele *et al.,* 2012), and there are complications associated with such procedures including electrolyte imbalance and excess mucus production (Pokrywczynska *et al.,* 2014). The existence of more suitable materials for bladder repair/replacement could reduce the need for these complex procedures and their associated complications. Such materials could take the form of synthetic materials or regenerative medicine, but would need similar mechanical properties to the natural bladder.

The aim of this study is to use Dynamic Mechanical Analysis (DMA) to measure the viscoelastic properties of *porcine* bladder tissue. Bladder tissue is a viscoelastic material but the exact bladder tissue stiffness response to a frequency sweep is unknown. Viscoelastic structures can be defined by storage (k') and loss stiffness (k''). Storage stiffness characterises the structure's ability to elastically store energy and loss stiffness characterises the structure's ability to dissipate energy that is lost due to the viscous processes occurring in the structure. Previous studies on the bladder have investigated stress relaxation (van Mastrigt *et al.*, 1981), elastic modulus (Dahms *et al.*, 1998) and also the cyclic stress-strain properties (Zanetti *et al.,* 2012). However, none have measured the viscoelastic properties over a frequency range. A detailed understanding of bladder viscoelasticty is vital for developing accurate computational models of the bladder and also for the development of replacement materials.

2. Materials and Methods

Ex vivo whole *porcine* bladders were supplied by Fresh Tissue Supplies (East Sussex, UK). The bladders were of mixed sex from pigs all under a year old. Once received they were wrapped in tissue paper, soaked in Ringer's solution (Oxoid Ltd, Basingstoke, UK), placed in heat sealed plastic bags and stored in a freezer at -40°C. Previous studies have shown that freezing and thawing does not affect the mechanical properties of biological tissues such as: vocal tissue (Chan & Titze, 2003),

ligaments (Woo *et al.,* 1986) and articular cartilage (Szarko *et al.,* 2010). When specimens were required for testing, bladders were defrosted at room temperature, soaked in Ringer's solution for around three hours and then dissected.

Two methods of tensile DMA were used in this study; the first made use of looped bladder samples and the second rectangular samples. The samples were initially prepared by dissecting the bladders using three cuts (Figure 1), made using surgical scissors (Fischer Scientific, UK). The dissection resulted in four areas of bladder: two looped central areas of the bladder, the dome region and the trigone region with ureters and urethra attached.

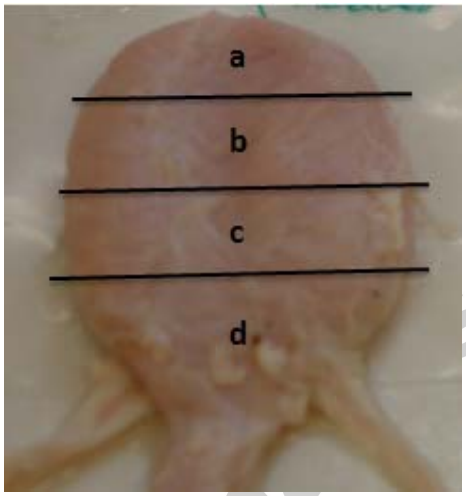

Figure 1 - Initial dissection lines of bladder samples. Dome region (a), two central regions (b & c) and the trigone region (d). Lines indicate where cuts were made.

Experimental samples were obtained from the two central regions of the bladder (areas b and c in Figure 1). For testing of the rectangular samples the central looped regions were dissected again with two more cuts, to create two strips from one looped sample. These cuts were always in the same anatomical location, laterally aligned to the ureters. The length, width and thickness of each specimen were then measured with Vernier callipers (Fischer Scientific, UK) by taking three measurements. Table 1 shows the mean and standard deviation of the dimensions of the prepared samples.

Table 1 - Dimensions (mean and standard deviation) of the looped and rectangular samples. 10 looped and 18 rectangular samples were tested.

	Loop			Rectangular		
	Length (mm)	Width (mm)	Thickness (mm)	Length (mm)	Width (mm)	Thickness (mm)
Mean	54.5	24.0	4.7	59.3	25.6	5.0
Std Deviation	4.8	2.8	1.1	9.9	3.9	1.1

The viscoelastic properties of the bladder samples were determined from DMA using a Bose Electroforce 3200 testing machine coupled with WinTest DMA software (Bose Corporation, Electroforce Systems Group, Minnesota, USA). Patel *et al.* (2008) have given full details of the Bose testing machine. Bose testing machines have been previously used to test a variety of biological

materials including cartilage, heart chordae, lumbar discs and bladder (Fulcher *et al.,* 2009; Millard *et al.,* 2011; Gadd & Shepherd, 2011; Zanetti *et al.,* 2012).

Custom-designed fixtures were manufactured to enable the testing of both looped and rectangular samples. Each fixture consisted of two identical but separate parts to fasten the bladder samples to the base and actuator of the testing machine (Figure 2). The fixtures for the looped samples consisted of two horizontal cylinders that the loops were secured around. The rectangular fixtures consisted of two horizontal grips that were supplemented with fine sandpaper. The grips were fastened to the sample by turning two horizontal screws, creating a compressive force on the top and bottom of the sample. Tensile preloads of roughly 10 N and 20 N were applied to the rectangular and looped samples, respectively. These preloads were necessary to ensure that samples remained on the fixtures during testing. The specimens were then allowed to relax for 2 minutes. The preloaded samples of bladder were covered with tissue paper soaked in Ringer's solution so that the specimen did not dehydrate during testing. This is consistent with procedures previously described by Öhman *et al.* (2009) and Wilcox *et al.* (2014) for testing tendons and heart chordae. Preliminary results showed that this did not affect the results of the testing.

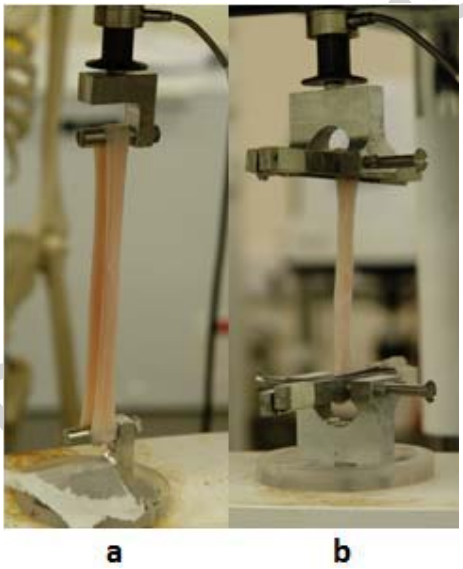

Figure 2 - Fixtures for testing the samples of bladder (a) looped samples; (b) rectangular samples.

A sinusoidally varying displacement was applied to the specimens between 2.5 mm and 5.5 mm. Both types of samples were tested in 11 steps with increasing frequencies: between 0.01 and 10 Hz (looped samples) and between 0.01 and 5 Hz (rectangular samples), as shown in Table 2. Maximum frequencies were chosen for both sample types as vibration of the bladder tissue adversely affected results at higher frequencies. Preliminary testing showed that the results were similar whether the frequency started at 0.01 Hz and was increased, or started at 5 or 10 Hz and decreased. Both the rectangular and looped samples were also subjected to 120 seconds of precycling at 5 and 10 Hz, respectively. During preliminary tests preconditioning loading cycles were found to be necessary to ensure that results from the initial frequency tested were comparable to

those obtained at subsequent frequencies. Therefore samples were preconditioned before testing. This is consistent with testing of other soft tissues (Öhman *et al.,* 2009; Wilcox *et al.,* 2014).

Table 2 - Testing frequencies for looped and rectangular bladder samples.

Testing Order	Looped Samples (Hz)	Rectangular Samples (Hz)
1	0.01	0.01
2	0.05	0.05
3	0.1	0.1
4	0.25	0.25
5	0.5	0.5
6	0.75	0.75
7	1	1
8	2.5	2
9	5	3
10	7.5	4
11	10	5

The WinTest software uses readings of force and displacement from the load cell and displacement transducer and from this dynamic stiffness (k*) and the phase angle (δ) are calculated. Dynamic stiffness is found using Fourier analysis to determine the ratio of peak load to peak displacement. The phase angle is also found using Fourier analysis to determine the phase difference between the load and displacement. Storage (k') and loss stiffness (k'') were then calculated from:

$$k' = k^* \cos \delta \quad (1)$$

$$k'' = k^* \sin \delta \quad (2)$$

All statistical analysis of the data was undertaken using Minitab (Version 15.1.20.0, Minitab Inc., Pennsylvania, USA). The significance of the curve fits generated for storage and loss stiffness were tested using regression analysis to generate p-values. If the p-value was less than 0.05 the curve fit of the relationship was significant.

3. Results

Figures 3 and 4 show three sample results from the looped and rectangular tests, respectively. All the storage stiffness results follow the same trend, with initially increasing storage stiffness with frequency and then a decrease at higher frequencies. The loss stiffness results follow the same gently linear increase with increasing frequency.

Figures 5 and 6 show the average results for looped and rectangular samples, respectively. In figure 5 (loop) the storage stiffness increased up to a stiffness of around 2 N/mm and then decreased at higher frequencies. The loss stiffness increased from 0.25 to 0.30 N/mm over the frequency range. In figure 6 (rectangular) the storage stiffness increased up to a stiffness of around 0.80 N/mm and then decreased at higher frequencies. The loss stiffness increases from 0.10 to 0.13 N/mm over the frequency range. The loss stiffness was lower than storage stiffness for all frequencies tested.

The trends for storage stiffness were described by two curve fits: a logarithmic fit (equation 3) from 0.01 to 1 Hz and a second order polynomial fit (equation 4) for the remainder of the frequency sweep. The values for coefficients A, B, C, D and E can be found in table 3. These curve fits showed a strong correlation with R^2 values of 0.77 and above (most between 0.9 and 1) and all had p-values of less than 0.05 showing that they were significant.

$$k' = Aln(f) + B \qquad \text{for } 0.01 < f < 1$$

(3)

$$k' = C\left(f^2\right) + D\left(f\right) + E \qquad \text{for } f \geq 1 \qquad (4)$$

where k' is the storage stiffness and f is the frequency.

The average storage stiffness curve fits for the looped samples were:

$$k' = 0.0455ln\left(f\right) + 1.951 \qquad \text{for } 0.01 < f < 1 \qquad (5)$$

$$k' = -0.0073\left(f^2\right) + 0.0529\left(f\right) + 1.9207 \qquad \text{for } 1 < f < 10 \qquad (6)$$

The average storage stiffness curve fits for the rectangular samples were:

$$k' = 0.0202ln\left(f\right) + 0.7912 \qquad \text{for } 0.01 < f < 1 \qquad (7)$$

$$k' = -0.0129\left(f^2\right) + 0.0293\left(f\right) + 0.7733 \qquad \text{for } 1 < f < 5 \qquad (8)$$

Both types of sample also exhibited the same trends for loss stiffness, which has been described by a linear fit (equation 9). The coefficients *F* and *G* can be found in table 3. These linear fits showed strong correlation with R^2 values of 0.63 and above (most between 0.85 and 0.93) and all had $p < 0.05$ showing that they were significant.

$$k'' = F\left(f\right) + G \qquad (9)$$

where k'' is the loss stiffness and f is the frequency.

The average loss stiffness curve fits for the looped (equation 10) and rectangular samples (equation 11) were:

$$k'' = 0.009\left(f\right) + 0.226 \qquad \text{for } 0.01 < f < 10 \qquad (10)$$

$$k'' = 0.0093\left(f\right) + 0.0984 \qquad \text{for } 0.01 < f < 5 \qquad (11)$$

Storage (E') and loss moduli (E'') can also be used to describe materials and they are calculated by dividing the relevant stiffness by the shape factor (Fulcher *et al.*, 2009). The shape factor for rectangular samples was calculated from:

$$S = wd / h \qquad (12)$$

where, w is width, d is depth and h is height (Menard, 2008).

For example, the storage stiffness of sample 13 at 0.01 Hz is 0.44 N/mm and the equivalent storage modulus (E') is 0.21 MPa. However stiffness is used to compare the results in this study as the looped samples are essentially structures and therefore modulus would be inappropriate.

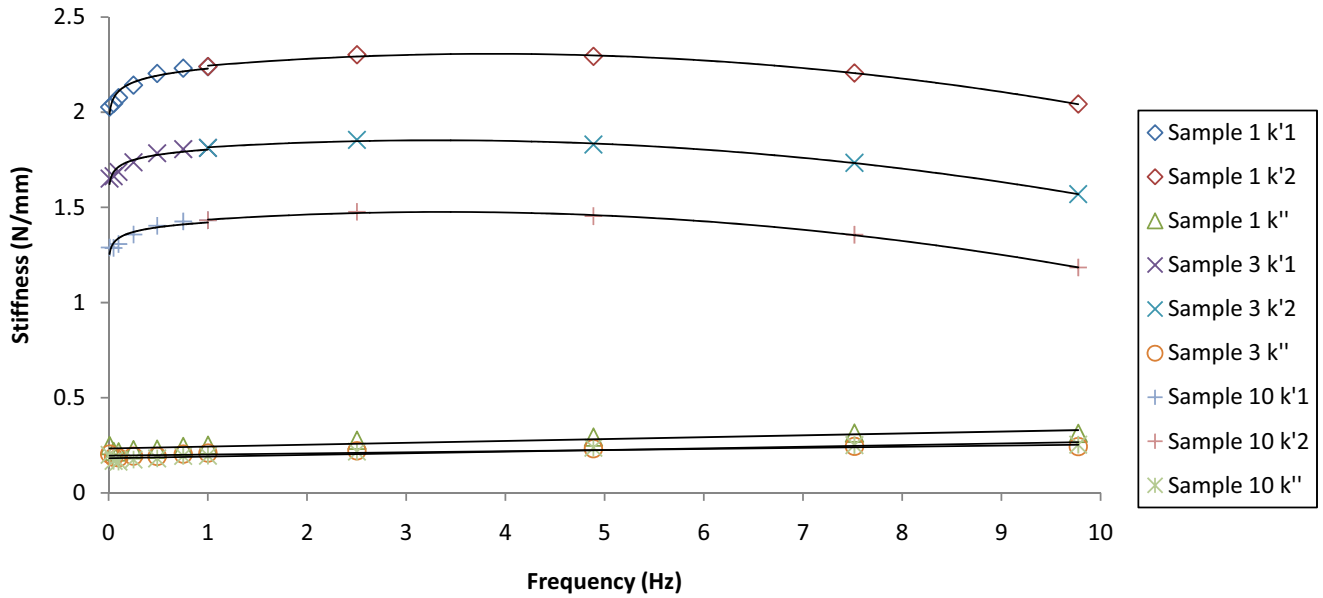

Figure 3 - Storage (k') and loss stiffness (k'') against frequency (f) for three individual looped samples. k'1 refers to the data points subjected to the first curve fit of storage stiffness up to 1 Hz (characterised by equation 3) and k'2 refers to the data points subjected to the second curve fit of storage stiffness up to the end testing frequency (characterised by equation 4). The loss stiffness (k'') curve fit is characterised by equation 9.

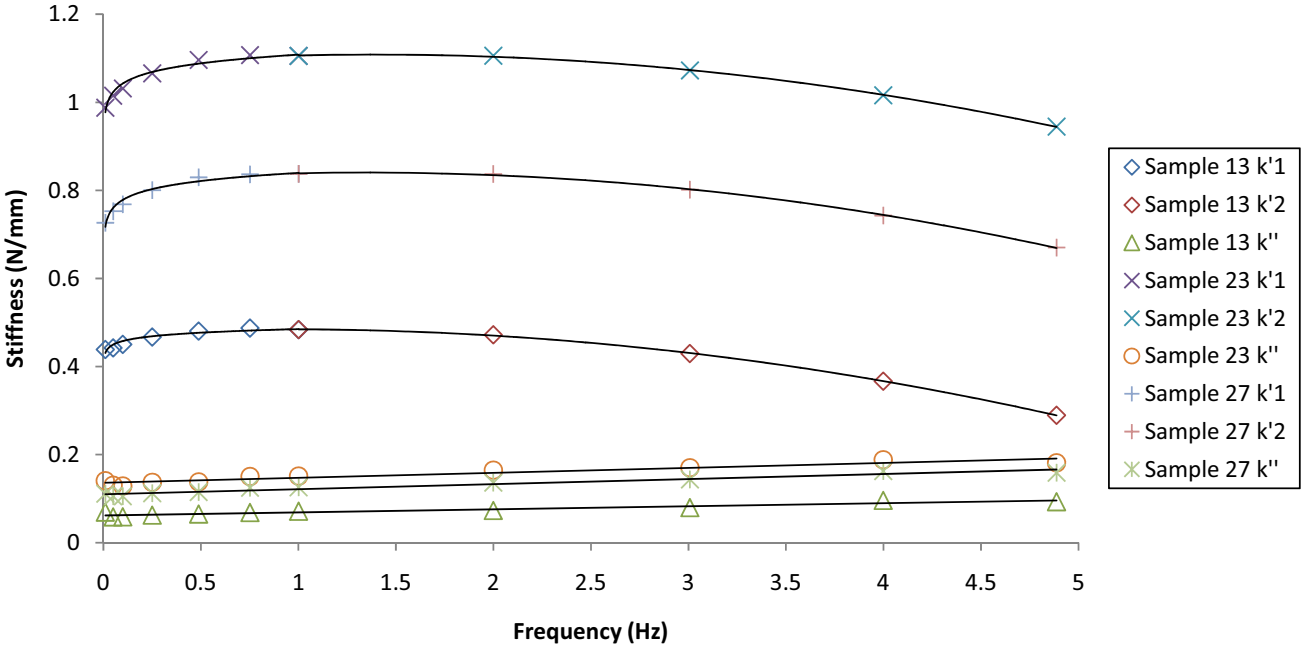

Figure 4 - Storage (k') and loss stiffness (k'') against frequency (f) for three individual rectangular samples. k'1 refers to the data points subjected to the first curve fit of storage stiffness up to 1 Hz (characterised by equation 3) and k'2 refers to the data points subjected to the second curve fit of storage stiffness up to the end testing frequency (characterised by equation 4). The loss stiffness (k'') curve fit is characterised by equation 9.

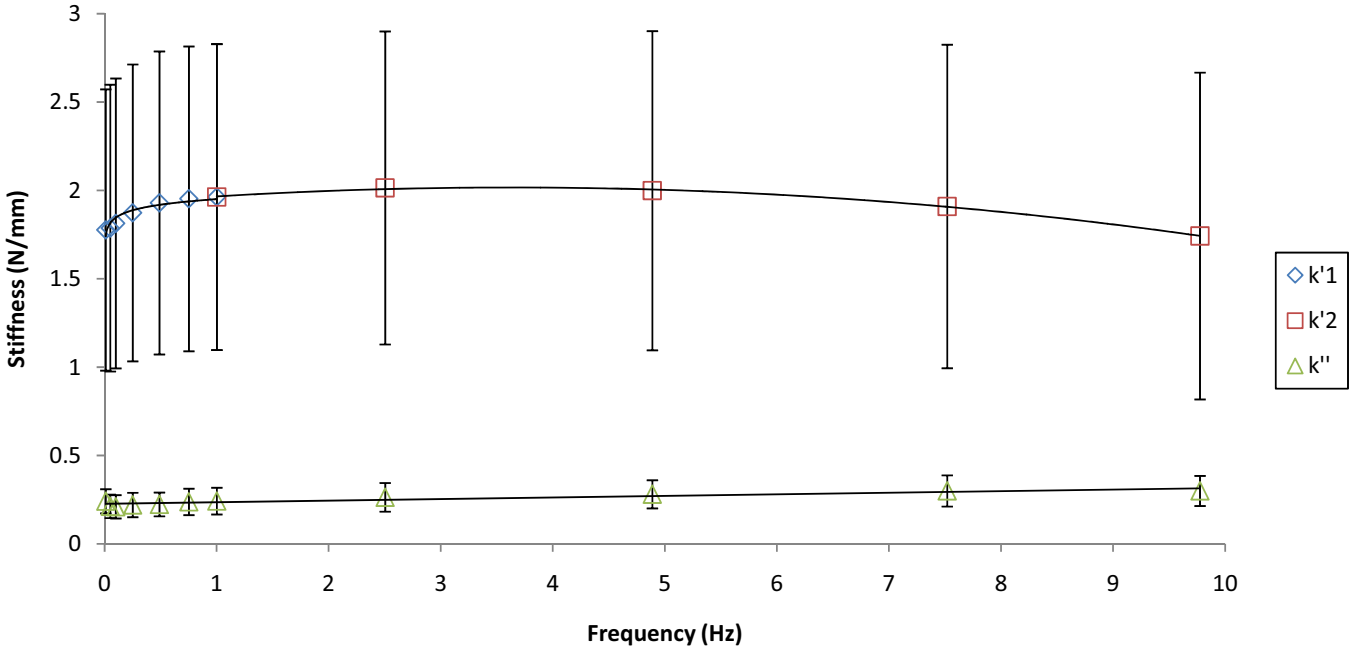

Figure 5 - Storage (k') and loss stiffness (k'') against frequency (f) for looped samples. Data points represent the average values, with one standard deviation error bars. k'1 refers to the data points subjected to the first curve fit of storage stiffness up to 1 Hz (described by equation 5) and k'2 refers to the data points subjected to the second curve fit of storage stiffness up to the end testing frequency (described by equation 6). The loss stiffness (k'') curve fit is described by equation 10.

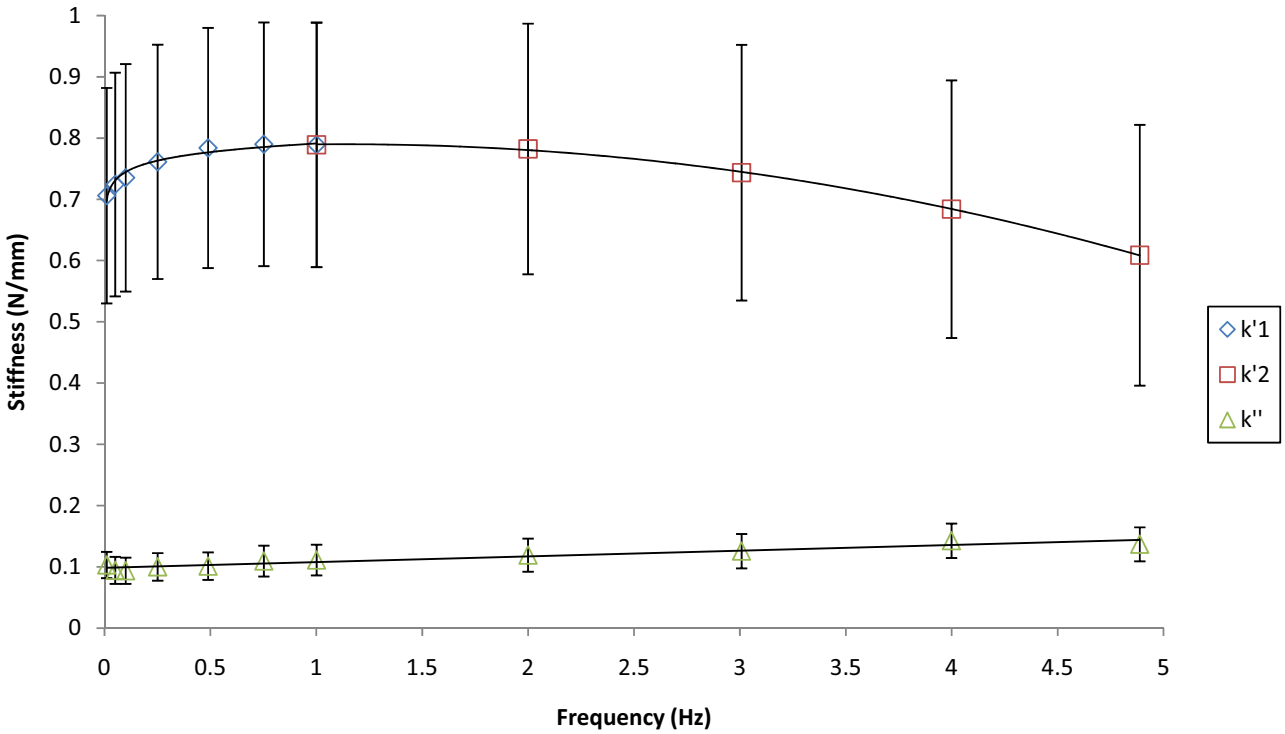

Figure 6 -Storage (k') and loss stiffness (k'') against frequency (f) for rectangular samples. Data points represent the average values, with one standard deviation error bars. k'1 refers to the data points subjected to the first curve fit of storage stiffness up to 1 Hz (described by equation 7) and k'2 refers to the data points subjected to the second curve fit of storage stiffness up to the end testing frequency (described by equation 8). The loss stiffness (k'') curve fit is described by equation 11.

Table 3 - Curve fit results for storage and loss stiffness. All coefficients were found to be statistically significant ($p < 0.05$).

Sample number	Sample type	Frequency range (Hz)	Storage stiffness (k') curve fit							Loss stiffness (k'') curve fit		
			0.01 to 1 Hz ($k'=A*\ln(f)+B$)			1 Hz to end frequency ($k'=Cf^2+Df+E$)				($k''=Ff+G$)		
			A	B	R^2	C	D	E	R^2	F	G	R^2
1	Loop	0.01 to 10	0.052	2.23	0.92	-0.0076	0.058	2.19	0.99	0.0099	0.23	0.86
2	Loop	0.01 to 10	0.039	1.44	0.91	-0.0070	0.047	1.41	0.99	0.0095	0.18	0.89
3	Loop	0.01 to 10	0.040	1.80	0.91	-0.0068	0.045	1.78	0.99	0.0059	0.20	0.85
4	Loop	0.01 to 10	0.031	1.41	0.8	-0.0067	0.042	1.39	0.99	0.0069	0.17	0.85
5	Loop	0.01 to 10	0.090	4.28	0.94	-0.0088	0.086	4.23	0.99	0.0143	0.40	0.84
6	Loop	0.01 to 10	0.042	1.65	0.89	-0.0071	0.049	1.62	0.99	0.0088	0.21	0.87
7	Loop	0.01 to 10	0.048	2.09	0.89	-0.0076	0.057	2.06	0.99	0.0100	0.28	0.86
8	Loop	0.01 to 10	0.041	1.60	0.9	-0.0071	0.048	1.57	0.99	0.0087	0.21	0.88
9	Loop	0.01 to 10	0.039	1.59	0.9	-0.0070	0.047	1.56	0.99	0.0074	0.19	0.84
10	Loop	0.01 to 10	0.036	1.42	0.86	-0.0071	0.048	1.39	0.99	0.0087	0.18	0.85
11	Rectangular	0.01 to 5	0.011	0.59	0.77	-0.0122	0.021	0.58	1.00	0.0071	0.08	0.63
12	Rectangular	0.01 to 5	0.011	0.69	0.84	-0.0125	0.022	0.68	1.00	0.0073	0.08	0.90
13	Rectangular	0.01 to 5	0.012	0.49	0.91	-0.0125	0.023	0.47	1.00	0.0069	0.06	0.90
14	Rectangular	0.01 to 5	0.012	0.44	0.95	-0.0112	0.015	0.44	0.99	0.0070	0.05	0.93
15	Rectangular	0.01 to 5	0.019	0.78	0.96	-0.0139	0.033	0.75	1.00	0.0102	0.11	0.92
16	Rectangular	0.01 to 5	0.023	0.78	0.96	-0.0133	0.035	0.76	1.00	0.0120	0.11	0.93
17	Rectangular	0.01 to 5	0.022	0.76	0.98	-0.0127	0.030	0.74	1.00	0.0103	0.10	0.92
18	Rectangular	0.01 to 5	0.021	0.80	0.98	-0.0129	0.027	0.79	1.00	0.0087	0.11	0.94
19	Rectangular	0.01 to 5	0.018	0.69	0.97	-0.0130	0.028	0.68	1.00	0.0088	0.09	0.93
20	Rectangular	0.01 to 5	0.017	0.61	0.97	-0.0125	0.025	0.60	1.00	0.0087	0.08	0.93
21	Rectangular	0.01 to 5	0.019	0.86	0.96	-0.0119	0.022	0.85	0.99	0.0087	0.11	0.89
22	Rectangular	0.01 to 5	0.021	0.76	0.97	-0.0132	0.029	0.74	1.00	0.0085	0.09	0.90
23	Rectangular	0.01 to 5	0.028	1.11	0.97	-0.0135	0.037	1.08	1.00	0.0113	0.14	0.91
24	Rectangular	0.01 to 5	0.029	1.03	0.98	-0.0135	0.038	1.01	1.00	0.0106	0.12	0.93
25	Rectangular	0.01 to 5	0.029	1.03	0.97	-0.0136	0.040	1.01	0.99	0.0112	0.13	0.91
26	Rectangular	0.01 to 5	0.028	1.17	0.96	-0.0136	0.039	1.14	1.00	0.0103	0.13	0.91
27	Rectangular	0.01 to 5	0.027	0.84	0.97	-0.0136	0.037	0.82	0.99	0.0115	0.11	0.95
28	Rectangular	0.01 to 5	0.018	0.80	0.94	-0.0128	0.028	0.79	1.00	0.0082	0.09	0.91

4. Discussion

The objective of this study was to investigate the viscoelastic properties of bladder tissue. Bladder tissue was found to be viscoelastic throughout the frequency range tested. Both the looped and rectangular bladder samples showed very consistent trends for storage and loss stiffness, where the same curve fits were used for both types of sample. Similar curve fits have been used in many other studies to describe viscoelastic properties; these include cartilage (Fulcher *et al.,* 2009) and heart chordae (Wilcox *et al.*, 2014). In this study the loss stiffness (k'') exhibited similar results throughout the frequency sweep with a near constant value over the frequency range; a similar tendency has been seen in other viscoelastic tissues such as articular cartilage (Fulcher *et al.*, 2009) and chordae tendineae from the heart (Wilcox *et al.*, 2014). Storage stiffness (k') however showed an initially increasing trend at low frequencies and then a decreasing trend at higher frequencies. Furthermore, the storage stiffness of the tissue did not change greatly throughout the test; the average minimum value was 82% of the maximum value. The findings for storage stiffness are in contrast to the same findings for cartilage (Fulcher *et al.,* 2009) and heart chordae (Wilcox *et al.*, 2014) where an increasing trend was found.

The range of frequencies tested varied from very low (0.01 Hz) to high (5 or 10 Hz) and this was intended to show the bladder response at physiological and traumatic conditions, respectively. The results indicate that the stiffness values at these frequencies were similar, with average storage stiffness values of 1.78 N/mm (low frequency), 1.74 N/mm (high frequency) and 0.71 N/mm (low frequency), 0.61 N/mm (high frequency) for looped and rectangular samples, respectively. It was expected that the results would be quite similar for the two different types of sample. This was because the looped samples, which had two load bearing structures, received a preload of 20 N and the rectangular samples, which had one load bearing structure, received a preload of 10 N. However, this was not the case as the storage stiffness results for the looped samples are more than two and a half times that of the rectangular samples.

The majority of previous mechanical testing of bladder studies have used rectangular samples to test a variety of bladder muscle uniaxially (Finkbeiner *et al.*, 1990; van Mastrigt *et al.*, 1978; Griffiths *et al.*, 1979; Alexander, 1971). However, testing of looped samples has been described in relation to bladder tissue by Alexander (1976) whilst testing series elasticity of rat bladders. Looped uniaxial testing was incorporated into this study to attempt to more closely imitate the function of the bladder but also to serve as a comparison for the rectangular uniaxial testing. It was shown that comparatively higher stiffness values were recorded in the looped samples and therefore multidirectional tension of bladder tissue stresses the tissue in a manner that makes it become stiffer and able to elastically store more energy. No other studies have compared the properties of bladder using the two methods described in this study.

Previous studies have found mechanical properties for rectangular transverse lateral sections of the bladder. Zanetti *et al.* (2012) found the secant modulus to be 0.1 - 0.45 MPa, Korossis *et al.* (2009) found the elastin phase slope to be 0.04 MPa and Dahms *et al.* (1998) found the elastic modulus as 0.26 MPa. All of these studies used static stress strain experiments. The average dynamic modulus for the rectangular samples for this investigation was 0.36 N/mm^2 which is comparable to the range found by Zanetti *et al* (2012). However, there are difficulties when comparing our results with material properties reported in the literature because viscoelastic properties are by definition

rate dependant. Therefore, comparisons made for the results obtained at different frequencies and strain rates can be misleading.

A study by Gilbert *et al.* (2008) states that collagen fibres, which are responsible for the mechanical response of the tissue, are predominantly aligned in the longitudinal direction. This may justify the low stiffness as the looped and rectangular samples were tested in the transverse direction. Furthermore bladder tissue has little elastin in any region of the bladder (Korossis *et al.,* 2009). Elastin stores the elastic energy of the material (Silver *et al.,* 2001) and the lack of elastin may also account for the low stiffness of the tissue.

If a similar study is performed on human bladder tissue the results of this study can be used to determine whether porcine bladders are a good comparison model. This has been done before with corneas and arteries (Zeng *et al.,* 2001; van Andel *et al.,* 2003). If the results from this study are validated by a human study then new urological procedures can be confidently tested on porcine bladders before being trialled in humans.

Any tissue engineered bladder tissue can also be compared to the values found in this study to determine if they have suitable viscoelastic properties. So far there has been clinical experience in implantation of tissue engineered bladders, albeit limited (Li *et al.,* 2014; Atla 2011). Some partial cystectomy procedures involve the use of *autologous* material as a replacement material for the bladder (Pokrywczynska *et al.,* 2014). The mechanical appropriateness of the small intestine and other *autologous* replacement tissues can now be tested. It is hoped that a material better suited to the role of replacement bladder for urine storage can be found without the associated adverse effects, such as excess mucus production and electrolyte imbalance (Pokrywczynska *et al.,* 2014).

Viscoelastic properties are also important for computer simulations of bladders such as Finite Element Analysis (FEA) or Computational Fluid Dynamics (CFD) studies which include bladder deformation. The correct viscoelastic properties need to be used for meaningful models. Previous CFD studies of the bladder have assumed the bladder wall to be rigid (Jin *et al.,* 2010) or have simulated the contracting detrusor muscle as fluid pressure (Pel *et al.,* 2007). For example, an application of this study into a CFD model could involve the investigation of tumour cell re-implantation during transurethral bladder tumour resection (Bryan *et al.,* 2010). Other simulations such as FEA modelling would be able to validate this study, if the results are comparable then FEA would be used to model the traumatic deformation of the bladder during a road traffic accident or find the allowable probing force during transurethral resection of bladder tumour surgery (TURBT).

One possible limitation of this study was the freezing of samples prior to testing. It is generally accepted that freezing does not influence the mechanical properties of biological materials. The majority of previous studies including tests on vocal tissue (Chan & Titze, 2003), ligaments (Woo *et al.,* 1986) and articular cartilage (Szarko *et al.,* 2010), state that there is no effect. However, other studies disagree with such findings, for example Venkatasubramanian *et al.* (2006) concluded that the freezing of *porcine* femoral arteries does affect its mechanical properties. Freezing technique is also important, Pelker *et al.* (1983) describe that freeze drying reduces the torsional strength of long rat bones when compared to freezing alone. As all samples tested in our current study underwent the same storage procedures, we would not expect the trends found to be affected by freezing.

5. Conclusions

The conclusions of this paper are as follows:

- Bladder tissue is viscoelastic through the range of frequencies tested, 0.01 to 5 or 10 Hz.
- The viscoelastic relationship changed with respect to frequency, where the average stiffness values were: 1.89 N/mm (storage) and 0.24 N/mm (loss) for the looped samples and 0.74 N/mm (storage) and 0.11 N/mm (loss) for the rectangular samples.
- Potential applications of these study findings include: enabling the use of *porcine* bladder as a comparable model to human bladder; comparisons to any tissue engineered or *autologous* bladder material; Finite Element Analysis and Computational Fluid Dynamic modelling of the bladder.

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