

# The dynamic viscoelastic characterisation and magnetic resonance imaging of poly(vinyl alcohol) cryogel

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1 *The Dynamic Viscoelastic Characterisation and Magnetic Resonance*  
2 *Imaging of Poly(Vinyl Alcohol) Cryogel: Identifying New Attributes and*  
3 *Opportunities*

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**Abstract**

Poly(vinyl alcohol) (PVA) cryogel is a biocompatible, synthetic hydrogel, compatible with magnetic resonance (MR) imaging . It is widely used as a biomaterial in tissue scaffolds and mimics to test various diagnostic techniques. The aim of this study is to characterise the effect of varying PVA concentration, molecular weight (MW) and manufacturing protocol on the viscoelastic mechanical properties and MR  $T_2$  relaxation time. Further to this MR imaging (MRI) was investigated as a method to quantify material homogeneity. Cylindrical samples of PVA, of varying MW, concentration and number of freeze thaw cycles (FTCs), were manufactured. Dynamic mechanical analysis was performed to evaluate the storage and loss moduli between frequencies of 0.5 and 10 Hz. MR  $T_2$  relaxation maps were imaged using a 7 T MRI instrument. Storage and loss moduli were shown to increase with MW, concentration, or the number of FTCs; with storage modulus ranging from 55 kPa to 912 kPa and loss modulus ranging from 6 kPa to 103 kPa. MR  $T_2$  relaxation time was shown to increase linearly with PVA concentration. The qualitative and quantitative heterogeneity of the PVA sample were identified through MR  $T_2$  relaxation time maps. Excitingly, PVA demonstrated a composition-dependent casual correlation between the viscoelastic mechanical properties and MR  $T_2$  relaxation time. In conclusion, this research thoroughly characterised the viscoelastic mechanical properties of PVA to support its extensive use as a biomaterial, and demonstrated the use of MRI to non-invasively identify sample heterogeneity and to predict the composition-dependent viscoelastic properties of PVA.

**KEYWORDS:** Biomaterials; MRI; Loss Modulus; PVA; Storage modulus.

## 42 ***1. Introduction***

43 Poly(Vinyl Alcohol) (PVA) is a widely used biomaterial [1, 2], particularly prevalent as a  
44 component of tissue scaffolds and a tissue mimicking material. The development of  
45 biomaterials, including PVA, for novel clinical applications offers significant social and  
46 economic impact. For example, connective tissue (e.g. arterial, articular cartilage and bone)  
47 damage impacts on the quality of life, and life expectancy of a large percentage of the world  
48 population [3, 4]. Cardiovascular Disease (CVD) was responsible for 164,000 deaths in the  
49 UK in 2019, and accounts for 27% of total deaths recorded [5]. Osteoarthritis (OA), a  
50 degenerative disease of synovial joints, is another prevalent example of connective tissue  
51 damage. In the USA, 1.5M hip and knee joint replacements were predicted for 2020,  
52 compared to 700,000 performed in the US in 2012 [6]. It is apparent that the clinical impact  
53 of connective tissue damage and disease is heavy and ever increasing. The surgical  
54 intervention, often required to repair or replace damaged tissue, poses an enormous and  
55 increasing cost to the worldwide economy. In 2017, cardiovascular disease cost the UK  
56 economy £28 billion [3]. The total cost of CVD in the USA was \$555 billion in 2016; in line  
57 with increasing population and obesity rates, this figure is projected to be \$1.1 trillion per  
58 year by 2035 [4]. PVA is also used as a tissue mimicking material. Tissue mimicking  
59 phantoms are an efficient and cost effective method of experimentally simulating the design,  
60 development and testing novel diagnosis or intervention techniques whilst avoiding the use of  
61 animal tissue. PVA is particularly well known as a vessel mimicking material and forms an  
62 essential tool in the research of cardiovascular biomechanics, diagnostic CVD techniques and  
63 medical device testing [7-9].

64 PVA cryogel consists of a mixture of PVA and water, it is physically cross-linked through  
65 one or more freeze-thaw cycles (FTCs). PVA cryogel is a biocompatible, synthetic, polymer  
66 with variable mechanical properties that depend on concentration, molecular weight, and the

67 temperature, duration, and number of FTCs during manufacture. This allows the material to  
68 replicate a broad-spectrum of soft tissues [10, 11], and has led to its frequent application as a  
69 component of heart valve stents [10], prostheses [9], intervertebral disc prostheses [12], as a  
70 material for articular cartilage replacement [13-17], and to model the mechanical properties  
71 of super-soft biological materials such as brain and lung tissue [18]. Furthermore, it is widely  
72 applied as a vessel mimicking material for cardiovascular phantoms [7, 19-21].

73 It is clear that PVA cryogels are used for numerous applications and possess the distinctive  
74 advantages that the mechanical properties can be designed through manufacturing. Yet, the  
75 extensive use of PVA, and ultimately the clinical translation, must be supported by relevant  
76 and thorough mechanical and chemical characterisation. To date, there are some key  
77 omissions in research literature which aim to characterise this biomaterial.

78 The majority of soft tissues in the body are viscoelastic [22-24], including connective tissue  
79 [25], therefore mechanical viscoelastic characterisation is crucial to underpin any research  
80 involving PVA tissue scaffolds or mimics. It has been reported in the literature that PVA  
81 exhibits viscoelastic properties [26-28], yet it has not yet been characterised using dynamic  
82 mechanical analysis (DMA). DMA measures dynamic viscoelasticity; characterising a  
83 material in terms of its ability to store energy (storage modulus) and its ability to dissipate  
84 energy (loss modulus). An induced stress results in instantaneous strain (the elastic response)  
85 and a time-dependent strain (the viscous response). This characterisation technique is  
86 analogous to in-vivo dynamic loading, and as such, is a crucial methodology to describe the  
87 mechanical response of the material.

88 PVA can be imaged by magnetic resonance (MR) imaging (MRI) using the nuclear magnetic  
89 resonance (NMR) signal of the water within the material. This is regarded as an important  
90 characteristic, particularly in its application as a vessel mimicking material [21, 29]. Chu et  
91 al. [29] demonstrated, for a fixed concentration, the change in MR  $T_1$  and  $T_2$  relaxation times

92 with FTCs. Orr et al. [30] showed the effect of PVA concentration and temperature on the  
93 MR  $T_1$  and  $T_2$  relaxation times, using a 3T MRI instrument. In these studies, the relationship  
94 between manufacturing parameters and MR relaxation is incomplete. This study will  
95 investigate MW, concentration and FTCs simultaneously, and thus, it will comprehensively  
96 support the dynamic mechanical analysis. Neither of these previous studies [29, 30] were able  
97 to confirm sample homogeneity, due to the lower spatial resolution, typical of clinical MRI  
98 instruments. This research will apply a high-resolution micro-imaging instrument (7 T) and  
99 thus determine the impact of the manufacturing protocol on the sample homogeneity.

100 In summary, this research will mechanically and chemically characterise PVA cryogel  
101 against compositional and manufacturing parameters, which to date have not been addressed  
102 in research literature. The study aims to characterise the effect of composition against MW  
103 and number of FTCs on the viscoelastic mechanical properties and the MR  $T_2$  relaxation time  
104 of PVA. Furthermore, this study will assess the change in the homogeneity of PVA samples  
105 during manufacture. The resulting viscoelasticity and MR  $T_2$  relaxation times are crucial to  
106 fully understanding the application of the biomaterial to tissue scaffolds and mimics, and  
107 underpin future clinical translation.

108

## 109 ***2. Methodology***

### 110 ***2.1 Sample Preparation***

111 Cylindrical specimens with dimensions of 20 mm diameter and 10 mm height were used for  
112 all DMA and MRI analyses. The specimen size was chosen to give the maximum surface area  
113 to assess the heterogeneity of PVA compositions through MR Imaging (relative to the core of  
114 the instrument). PVA was acquired from Sigma Aldrich (St Louis, Missouri, USA) and had a  
115 hydrolysis of 99+%. To incorporate the range of molecular weights (MW) of PVA this study  
116 utilised two different MWs; 89-98 kDa and 146-186 kDa. Each MW was manufactured at

117 concentrations of 10, 15, 17.5, and 20 %. A total of 72 samples (six samples for each  
 118 composition of PVA) were used for mechanical testing; with another 36 (three samples per  
 119 composition of PVA) samples used for imaging.

120 *Table 1: Reference table for molecular weight, freeze thaw cycles, and concentrations of PVA compositions used in this*  
 121 *study.*

Composition reference	Molecular Weight (kDa)	No. Freeze thaw cycles	Concentrations (% w/w)
PVA-A	89-98	3	10
			15
			17.5
			20
PVA-B	146-186	1	10
			15
			17.5
			20
PVA-C	146-186	3	10
			15
			17.5
			20

122  
 123 PVA powder was stirred into distilled water, covered (to reduce evaporation) and stirred  
 124 using an automatic overhead stirrer, at 90 °C for one hour, where upon it was fully dissolved.  
 125 The mixture was stirred for another 1 hour, to retain homogeneity, whilst the solution was  
 126 allowed to cool down to room temperature. The solution was poured into cylindrical moulds  
 127 and then underwent 3 FTCs. The FTC process consisted of three phases across 24 hours; 12  
 128 hours in the freezer at -20°C, 4 hours in the freezer without active cooling (to increase  
 129 temperature gradually), and finally 8 hours at a room temperature of 22 ± 1 °C. After the  
 130 required FTCs, the samples were stored in distilled water for three days to reach hydrostatic  
 131 equilibrium. Each MW and all concentrations were assessed after 3 FTCs. Molecular weight  
 132 146-186 kDa was assessed at 1 FTC. Molecular weight 89-98kDa was not assessed at 1 FTC  
 133 due to low stiffness.

134 **2.2 Experimental Protocol**

135 **2.2.1 Magnetic Resonance Imaging**

136 MR images of 3 PVA samples at four different concentrations of PVA-A, PVA-B, and PVA-  
137 C, were collected using a Bruker Biospin DMX-300 spectrometer (Bruker UK Limited,  
138 Coventry, UK) operating at a proton resonance frequency of 300.13 MHz. A Micro 2.5  
139 imaging probe, with a 25 mm <sup>1</sup>H quadrature Radio Frequency (RF) coil, was used for all  
140 experiments. MR  $T_2$  relaxation maps were determined from a series of 8 echo images using  
141 the spin-echo imaging sequence RARE (Rapid Acquisition with Relaxation Enhancement)  
142 [30]. Images were collected using a 128 × 128 pixel array, 25 × 25 mm<sup>2</sup> field of view and a  
143 slice thickness of 1 mm. MR  $T_2$  relaxation maps were calculated using a repetition time ( $T_R$ )  
144 of 15,000 ms, with a RARE factor of 8.

145 To quantify the difference between the number of FTCs, and storage in water on the MR  $T_2$   
146 relaxation time of PVA, nine samples of 10% 146-186 kDa PVA were imaged immediately  
147 post-thawing, after 1 and 3 FTCs without storage in water (*Figure 1*). They were then  
148 allowed to reach equilibrium in distilled water for three days, and subsequently imaged again.  
149 These samples were not mechanically tested in order to remove any additional variables  
150 which may alter their MR  $T_2$  relaxation time.

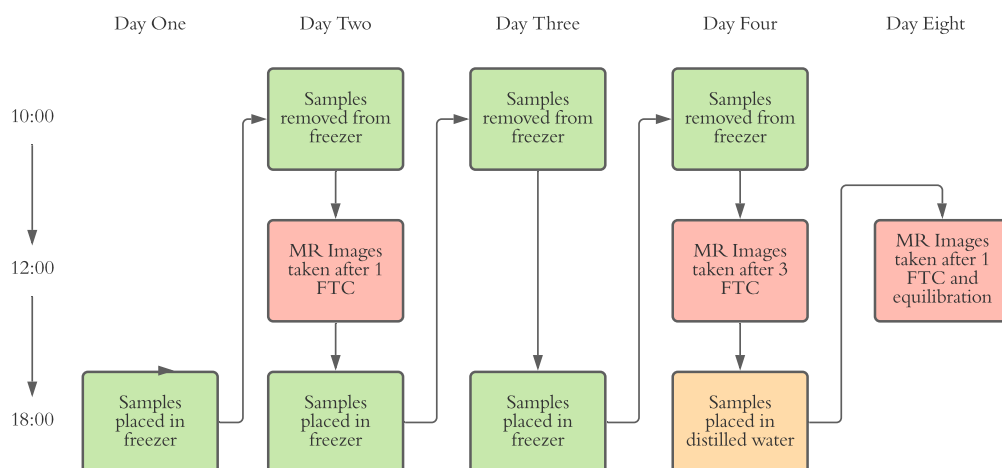


Figure 1: Flow-chart showing protocol used to assess the effect of 3 repeat freeze thaw cycle and equilibration on  $T_2$  relaxation time

151 **2.2.2 Dynamic Mechanical Analysis**



152 A Bose Electroforce 3200 (Bose Corporation, ElectroForce systems Group, Minnesota, USA;  
 153 now TA Instruments, Delaware, USA) mechanical testing machine, running Wintest software  
 154 (TA Instruments, Delaware, USA) was used to perform all DMA. A sinusoidal displacement  
 155 ( $d$ ) was applied to the samples, and corresponding load ( $F$ ) and phase lag ( $\delta$ ) were  
 156 measured. A Fast Fourier Transform (FFT) of both the load ( $F^*$ ) and displacement ( $d^*$ ) was  
 157 then performed, with the ratio between their respective data-set lengths giving a dynamic  
 158 stiffness ( $K^*$ ); equation 1. The storage ( $k'$ ) and loss ( $k''$ ) stiffness were then calculated  
 159 using equations 2 and 3. The sample diameter ( $D$ ), and height ( $h$ ) were measured for all  
 160 samples in order to calculate their shape factor ( $S$ ); which for a cylinder is given by equation  
 161 4. The storage ( $E'$ ) and loss ( $E''$ ) moduli were then calculated using equations 5 and 6,  
 162 respectively. This method is consistent with characterising the dynamic viscoelasticity of  
 163 natural and synthetic biomaterials [22, 24].

$$164 \quad K^* = \frac{F^*}{d^*} \quad (\text{Eq. 1})$$

$$165 \quad k' = k^* \cos(\delta) \quad (\text{Eq. 2})$$

$$166 \quad k'' = k^* \sin(\delta) \quad (\text{Eq. 3})$$

$$167 \quad S = \frac{\pi D^2}{4h} \quad (\text{Eq. 4})$$

$$168 \quad E' = \frac{k'}{S} \quad (\text{Eq. 5})$$

$$169 \quad E'' = \frac{k''}{S} \quad (\text{Eq. 6})$$

170 Samples were compressed by 20% of their measured height using a frequency sweep of 0.5 –  
 171 10 Hz. This frequency range was chosen to replicate the strain rates expected in different  
 172 connective tissues; such as coronary arteries (0.5-10 Hz) [24], heart valve leaflets (0.5-10 Hz)  
 173 [31], chordae tendineae (0.5 – 10 Hz)[32], and articular cartilage under physiological loading  
 174 (1 – 10 Hz) [33]. A preconditioning cycling of 5 Hz for 60 seconds was run prior to each test

175 to negate the effects of stress relaxation. Complex moduli can be calculated from storage and  
176 loss moduli using equation 7 (note it is not included in the results or supplementary sections  
177 to avoid repetition of data).

$$178 \quad E^* = \sqrt{E'^2 + E''^2} \quad (\text{Eq. 7})$$

### 179 **2.2.3 Data Analysis**

180 All statistical analyses were performed using SigmaPlot (SYSTAT, San Jose, CA, USA).

181 Data has been represented as mean  $\pm$  standard deviation (SD) unless otherwise stated.

182 Regression analysis has been used to empirically fit trendlines for comparisons between  
183 viscoelastic properties (storage,  $E'$  and loss,  $E''$  moduli) and concentration (equations 8 and  
184 9); MR  $T_2$  relaxation rate, MR  $R_2$  and concentration (equation 10); and viscoelastic properties  
185 and MR  $T_2$  relaxation rate (equations 11-14). All the constants of the equations for regression  
186 and coefficients of determination ( $R^2$ ) for trend lines are given in supplementary data.

$$189 \quad E', E'' = a + be^{dC} \quad (\text{Eq. 8})$$

187 Where  $a_s$ ,  $b_s$ , and  $d_s$  are constants for  $E'$ ; and  $a_l$ ,  $b_l$ , and  $d_l$  are constants for  $E''$ ; and  $C$  is  
188 the concentration of PVA.

$$190 \quad E' = f + gC \quad (\text{Eq. 9})$$

$$191 \quad R_2 = x + yC \quad (\text{Eq. 10})$$

$$192 \quad E'_A = a_s + b_s e^{d_s R_2} \quad (\text{Eq. 11})$$

$$193 \quad E'_B = a_s + b_s R_2. \quad (\text{Eq. 12})$$

$$194 \quad E''_A = a_l + b_l e^{d_l R_2} \quad (\text{Eq. 13})$$

$$195 \quad E''_B = a_l + b_l R_2. \quad (\text{Eq. 14})$$

196 A one way ANOVA was used to ascertain whether a statistically significant difference  
197 between the loss moduli of 15, 17.5 and 20% PVA-C was present. Results with  $p < 0.05$  were  
198 considered significant.

199

200 **3. Results**

201 **3.1 Dynamic viscoelasticity and composition of PVA**

202 **3.1.1 PVA Concentration and viscoelastic properties**

203 **Storage Modulus**

204 An increase in PVA concentration from 10-20% resulted in an increase in Storage modulus at  
205 all frequencies (Figure 2); this was the case for the three compositions of PVA (A, B, C). For  
206 example, for PVA-A tested at 0.5 Hz, the storage modulus increased from  $158 \pm 9.5$  kPa at  
207 10% concentration to  $484 \pm 24$  kPa at 20% concentration. Frequency affected all  
208 compositions in a similar manner, with an increase in storage modulus as the frequency was  
209 increased. For PVA-A, between 0.5 and 10 Hz, average increases of 8.2, 8.1, 15, and 13%  
210 were calculated for 10, 15, 17.5, and 20% concentrations.

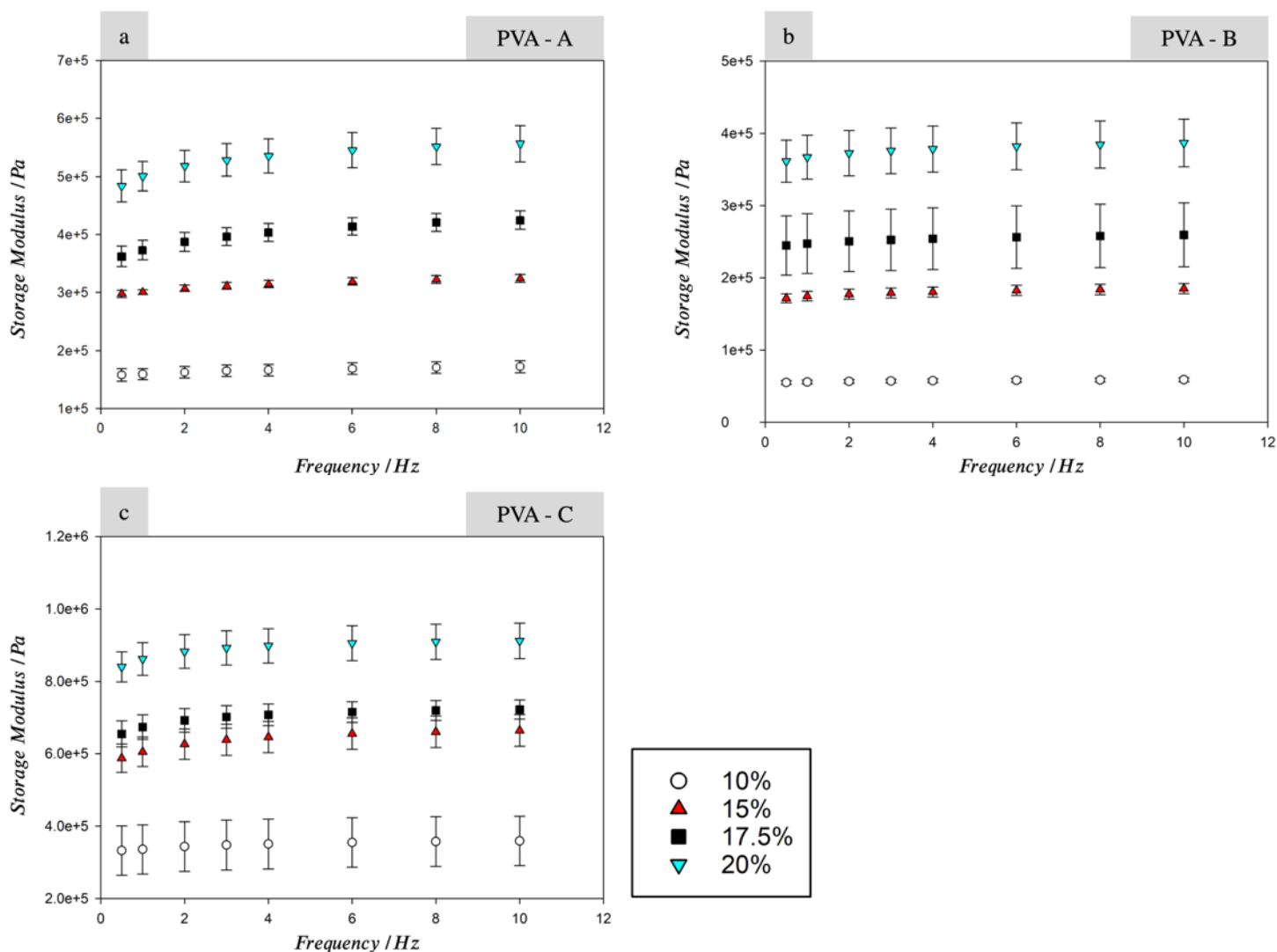


Figure 2: Storage modulus for PVA-A (a), PVA-B (b), and PVA-C (c), at concentrations of 10, 15, 17.5 and 20% w/w. Error bars show 95% confidence intervals ( $n = 6$ ).

211 An exponential relationship was evident between storage modulus and concentration for  
 212 PVA-A ( $R^2 = 0.99$ ) and PVA-B ( $R^2 = 0.96$ ) (Figures 3a and 3b). This differed to the linear  
 213 relationships shown by PVA-C ( $R^2 = 0.94$ ) (Figure 3c). These relationships were apparent for  
 214 all frequencies.

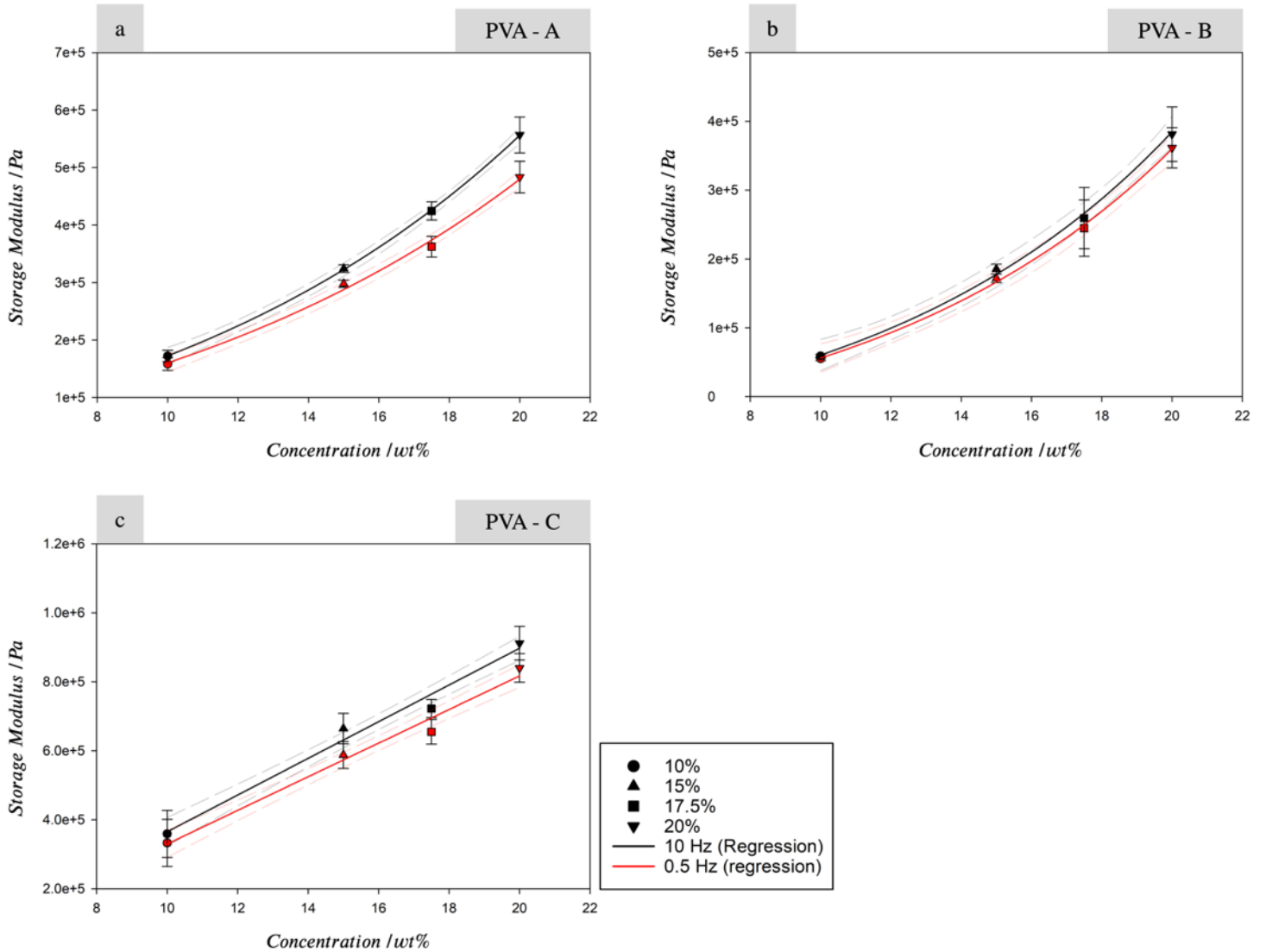


Figure 3: Storage Modulus plotted against concentration for 0.5 Hz (red), and 10 Hz (black) for PVA-A (a), PVA-B (b), and PVA-C (c). Error bars show 95% confidence ( $n=6$ ). Regression lines for 0.5 Hz (red), and 10 Hz (black) are also given; dashed lines show 95% confidence intervals for regression.

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219 **Loss Modulus**

220 For all samples, loss modulus was found to be lower than storage modulus. For PVA-A and  
 221 B, a similar relationship was seen between loss modulus and PVA concentration, with an  
 222 increase in concentration resulting in an increase in loss modulus at all frequencies tested  
 223 (Figure 4). For example, for PVA-A tested at 0.5 Hz, an increase from  $25.4 \pm 2.3$  kPa at 10%  
 224 concentration to  $86.5 \pm 2.3$  kPa at 20% concentration was measured. A large increase in loss  
 225 modulus was seen at all frequencies between 10 and 15% concentration for PVA-C  
 226 (increasing from  $38.1 \pm 5.4$  kPa to  $87.8 \pm 11$  kPa at 0.5 Hz, and  $39.9 \pm 3.0$  kPa to  $73.1 \pm 13$   
 227 kPa at 10 Hz). However, no statistically significant difference was seen between 15, 17.5 and  
 228 20% at any frequency ( $p = 0.53 \pm 0.25$ ) (Figure 4).

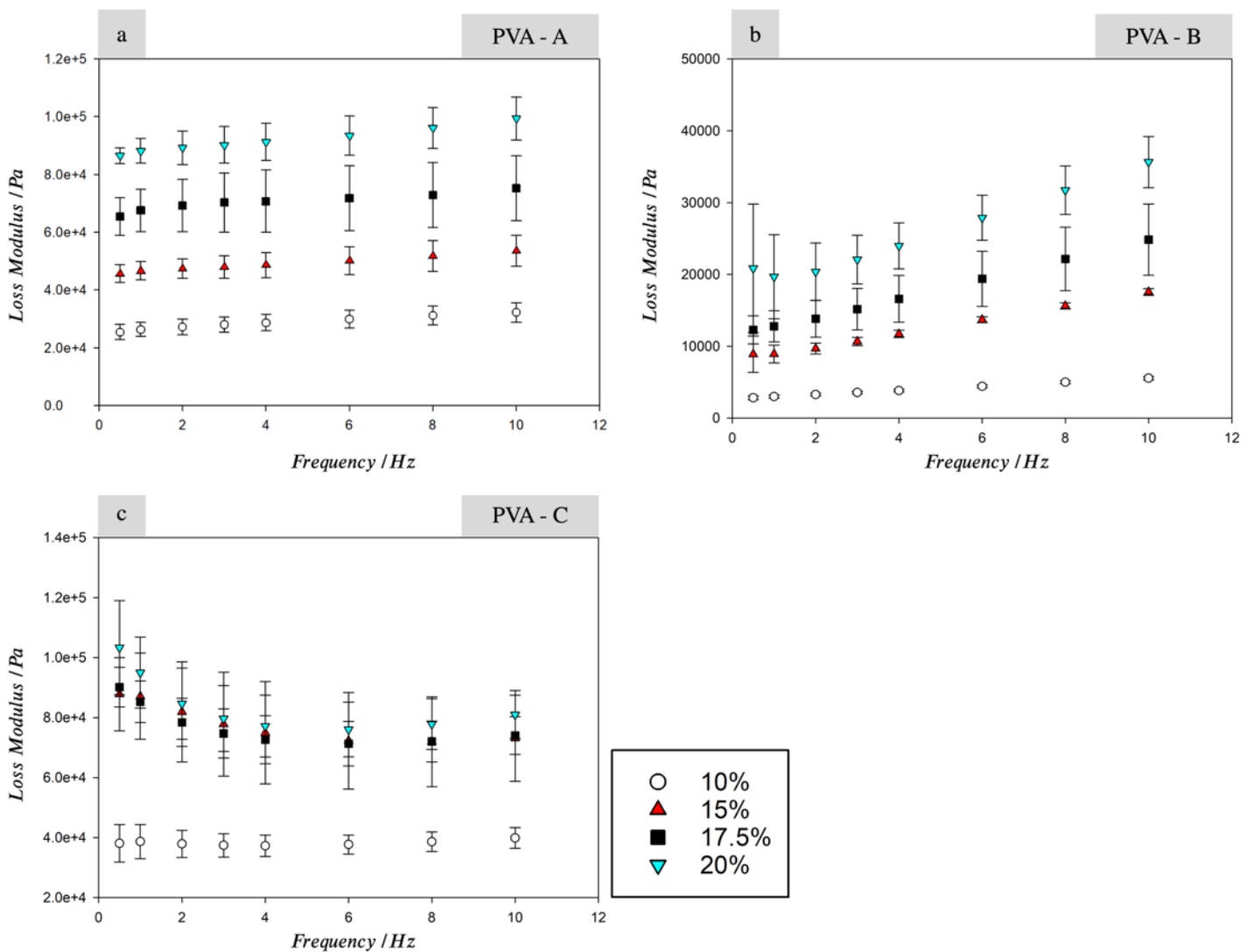


Figure 4: Loss modulus for PVA-A (a), PVA-B (b), and PVA-C (c), at concentrations of 10, 15, 17.5 and 20% w/w. Error bars show 95% confidence intervals ( $n = 6$ ).

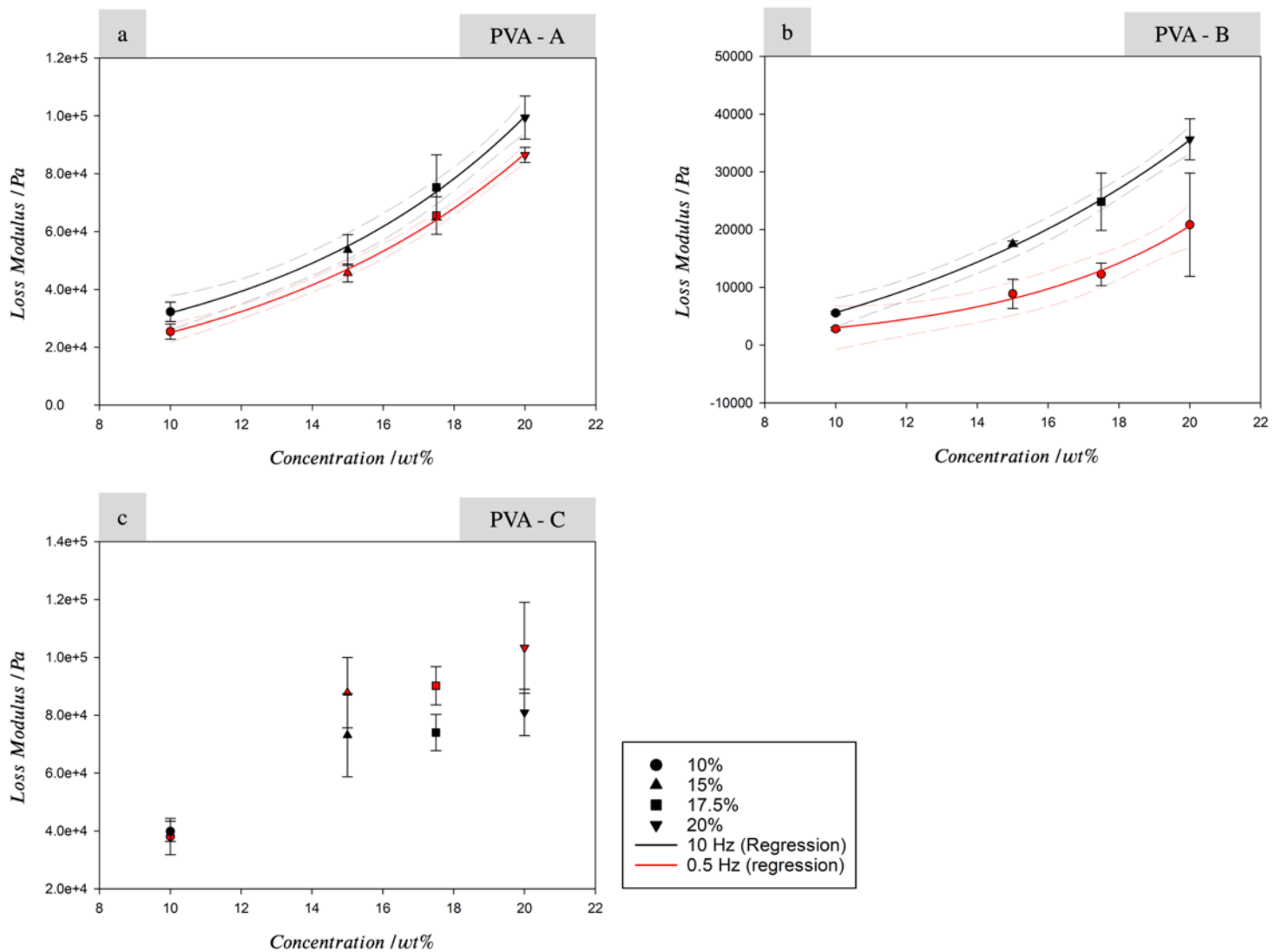


Figure 3: Loss Modulus plotted against concentration for 0.5 Hz (red), and 10 Hz (black) for PVA-A (a), PVA-B (b), and PVA-C (c). Error bars show 95% confidence ( $n=6$ ). Regression lines for 0.5 Hz (red), and 10 Hz (black) are also given; dashed lines show 95% confidence intervals for regression.

230 Frequency was shown to impact loss modulus; for PVA-A between 0.5 and 10 Hz, average  
 231 changes of 22%, 4.8 %, 10%, and 7.8% were seen at 10 ,15, 17.5, and 20% w/w, respectively.  
 232 A more consistent increase of 49, 49, 50, and 41% at each concentration was seen for PVA-  
 233 B. For PVA-C, a change of 0.1% was seen at 10%, and a decrease of 20, 21, and 27% was  
 234 seen for 15, 17.5, and 20% w/w, respectively. An exponential relationship was derived  
 235 empirically between the loss modulus and concentration for PVA-A ( $R^2 = 0.95$ ) and PVA-B

236 ( $R^2 = 0.91$ ) (Figures 5a and b). However, a similar relationship was not observed for PVA-C  
237 (Figure 5c), thus no trend line has been empirically fitted.

### 238 ***3.1.2 Molecular weight of PVA and viscoelastic properties.***

239 The Storage modulus increased for all concentrations of PVA with increasing molecular  
240 weight, from 89-98 kDa to 146-186 kDa (Figures 2a and 2c). This increase was observed for  
241 all frequencies, with an average of a  $2.1 \times$  increase at 10% w/w concentration;  $2.0 \times$  at 15%  
242 w/w concentration;  $1.8 \times$  at 17.5% concentration; and  $1.7 \times$  at 20% concentration. As stated  
243 in section 3.1.1, the frequency dependency of storage modulus showed little change, and both  
244 compositions could be empirically characterised using an exponential trend line to compare  
245 storage modulus and PVA concentration (Figures 3a and 3c).

246 An increase in loss modulus was also observed for 10, 15, and 17.5% at all frequencies. An  
247 average increase of  $1.3 \times$  at 10% w/w concentration;  $1.6 \times$  at 15% w/w concentration;  $1.1 \times$   
248 at 17.5% concentration. A decrease of  $0.91 \times$  was noted for 20% w/w concentration. As per  
249 section 3.1.1, the frequency dependency of the loss modulus was shown to be different for the  
250 two MWs of PVA, with the loss modulus increasing with respect to frequency for PVA-A,  
251 and decreasing for PVA-C. It was also previously noted that no significant trend could be  
252 used to describe the effect of concentration on loss modulus for the higher MW PVA, as  
253 compared to lower MW PVA concentrations where the trend could be empirically  
254 characterised using an exponential trend line.

### 255 ***3.1.3 Freeze thaw cycles on viscoelastic properties.***

256 The storage modulus increased for all concentrations of PVA with an increase from 1 to 3  
257 FTCs (Figures 2b and 2c). This increase was observed for all frequencies, with an average  $6.1$   
258  $\times$  increase at 10% w/w concentration;  $3.5 \times$  at 15% w/w concentration;  $2.8 \times$  at 17.5%  
259 concentration; and  $2.4 \times$  at 20% concentration.

260 An increase in loss modulus was also observed for all samples at all frequencies when the  
 261 number of FTCs was increased. However, smaller increases were seen at higher frequencies  
 262 for each concentration. This decrease varied from  $14 \times$  at 0.5 Hz to  $7.2 \times$  at 10 Hz at 10%  
 263 w/w concentration;  $9.9 \times$  to  $4.2 \times$  at 15% w/w concentration;  $7.3 \times$  to  $3 \times$  at 17.5% w/w  
 264 concentration; and  $4.9 \times$  to  $2.3 \times$  at 20% w/w concentration. The inter sample variation  
 265 increased for 10% PVA when the number of FTCs was increased to 3. With SD being  $2.8 \pm$   
 266  $0.08\%$  of the mean after 1 FTC, and  $17 \pm 0.4\%$  of the mean after three cycles.

### 267 **3.2 MRI and composition of PVA**

#### 268 **3.2.1 Molecular Weight, Concentration and MR $T_2$ Relaxation Time.**

269 MR  $T_2$  Relaxation time was shown to decrease as concentration of PVA was increased from  
 270 10 to 20% (Table 2). The plot of MR  $T_2$  relaxation rate against concentration for all  
 271 compositions shows a linear relationship (Figure 6). The difference in MW (comparing PVA-  
 272 A and PVA-C), created a minimal difference in MR  $T_2$  relaxation times  $\leq 5\%$ . Comparing  
 273 PVA-B and PVA-C showed that as the concentration was increased, the difference between  
 274 the MR  $T_2$  relaxation time increased with FTC.

275 *Table 2: MR  $T_2$  Relaxation times (mean  $\pm$  SD) for PVA-A, B, and C, at concentrations of 10, 15, 17.5 and 20 % w/w.*

%w/w	PVA-A		PVA-B		PVA-C		Increase in MW (A-C)	Increase in FTC (B-C)
	Mean (ms)	SD	Mean (ms)	SD	Mean (ms)	SD	% Mean difference	
10	117	0.8	119	5.2	123	3.6	5.1	3.4
15	90.2	1.1	86.0	2.6	90.3	1.0	0.1	5.0
17.5	82.8	0.4	70.0	1.5	82.2	1.3	-0.7	17.4
20	77.9	1.0	59.1	0.7	78.0	0.1	0.1	32.0



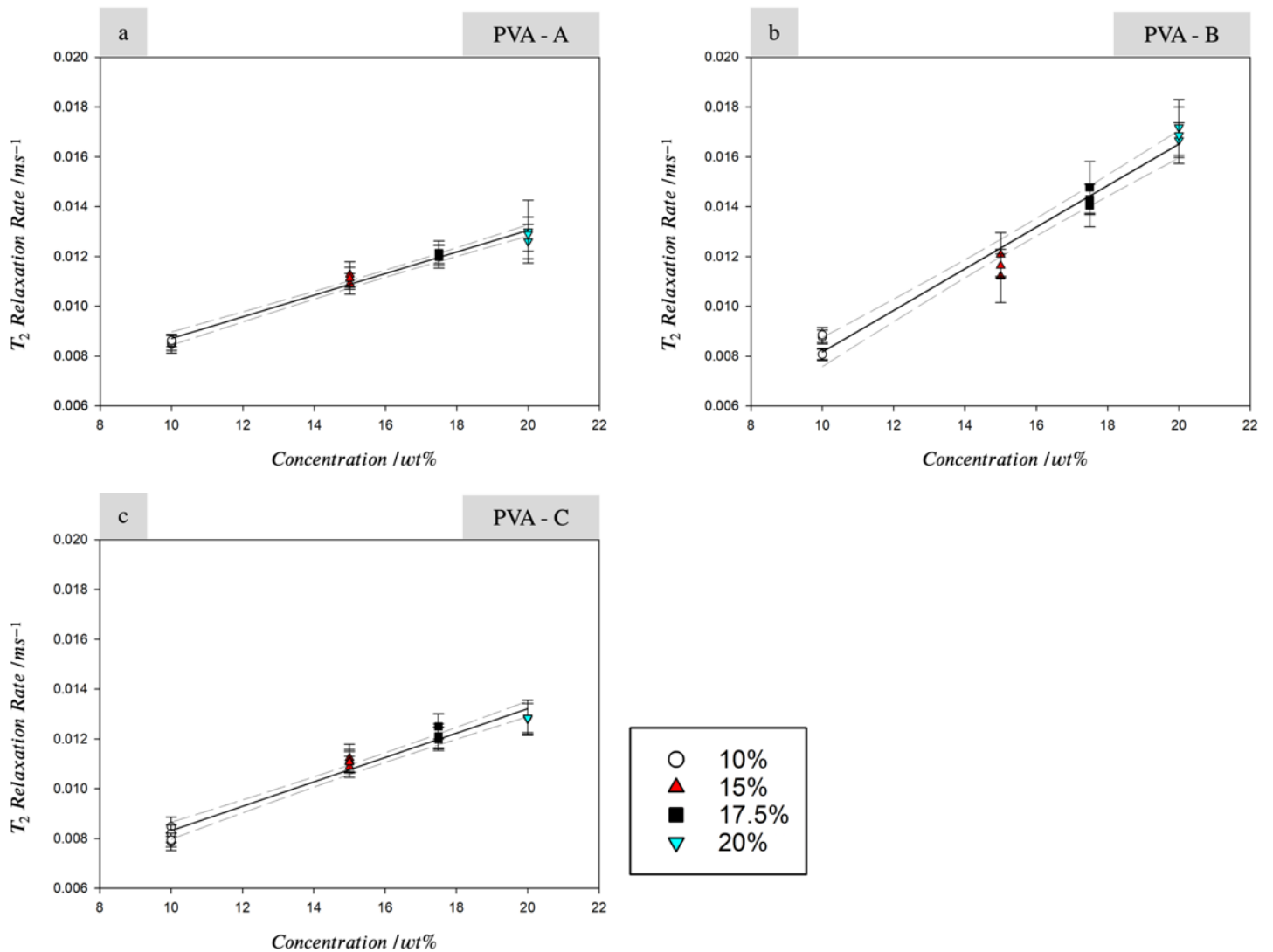


Figure 6: MR  $T_2$  Relaxation Rate vs. concentration for PVA-A (a), PVA-B (b), and PVA-C (c). Points show average  $T_2$  Relaxation Rate for each sample. Error bars show intra-sample standard deviation. Dashed lines show 95% confidence intervals for regression.

277

### 278 3.2.2 Freeze thaw cycles and MR $T_2$ relaxation time.

279 An increase in the number of FTCs led to an increase in the mean MR  $T_2$  relaxation time of a  
 280 sample, increasing from  $101 \pm 5.5$  ms after one FTC, to  $109 \pm 8.6$  ms after three FTCs (Table  
 281 3). A further increase to  $122 \pm 5.7$  ms was seen after three days of equilibration in distilled  
 282 water. No clear trend can be seen when analysing the change in inter-sample SD of the mean  
 283 with FTCs.

284 Figure 7 qualitatively demonstrates that the number of FTCs and further equilibrium altered  
 285 the heterogeneity of samples. This observation is quantified by the increase in the mean intra  
 286 sample standard deviation from 4.1% of the mean after 1 FTC, to 8.1% after 3 FTCs, and to  
 287 10.6% after equilibration (Table 3). This increase in the intra-sample variation can also be  
 288 seen when analysing the distribution of MR  $T_2$  relaxation times for all pixels within each  
 289 relaxation map (Figure 8). The pixel distributions from three of the nine samples after 1 and 3  
 290 FTCs and after equilibration, are displayed, and a broader peak in MR  $T_2$  distribution after 3  
 291 FTCs (Fig. 8b) can be observed, compared to the distribution after 1 FTC (Fig. 8a).  
 292 Table 3 shows the measurement of the sample cross-section from the MR  $T_2$  relaxation map  
 293 as an indicator of the change in sample shape after each step. This showed a decrease in area  
 294 between 1 and 3 FTCs from  $298 \pm 6 \text{ mm}^2$  to  $275 \pm 4 \text{ mm}^2$ , and a further decrease to  $246 \pm 4$   
 295  $\text{mm}^2$  after three days of equilibration.

296 *Table 3: MR  $T_2$  Relaxation times for 10% w/w PVA at 1 and 3 freeze cycles, and after a further 3 days of*  
 297 *equilibration. (n = 9 samples)*

No. Freeze thaw cycles	Mean (ms)	Inter-sample SD of Means (ms)	Inter-sample SD of means (% of mean)	Mean of Intra-sample SDs (ms)	Mean Intra-sample SD (% of mean)	Mean $\Delta$ Cross-sectional area ( $\text{mm}^2$ )
1 (equivalent to PVA-B)	101	5.5	5.4	4.1	4.1	$298 \pm 6$
3 (equivalent to PVA-C)	109	8.6	7.9	8.8	8.1	$275 \pm 4$
3 + Equilibration	122	5.7	4.8	12.9	10.6	$246 \pm 4$

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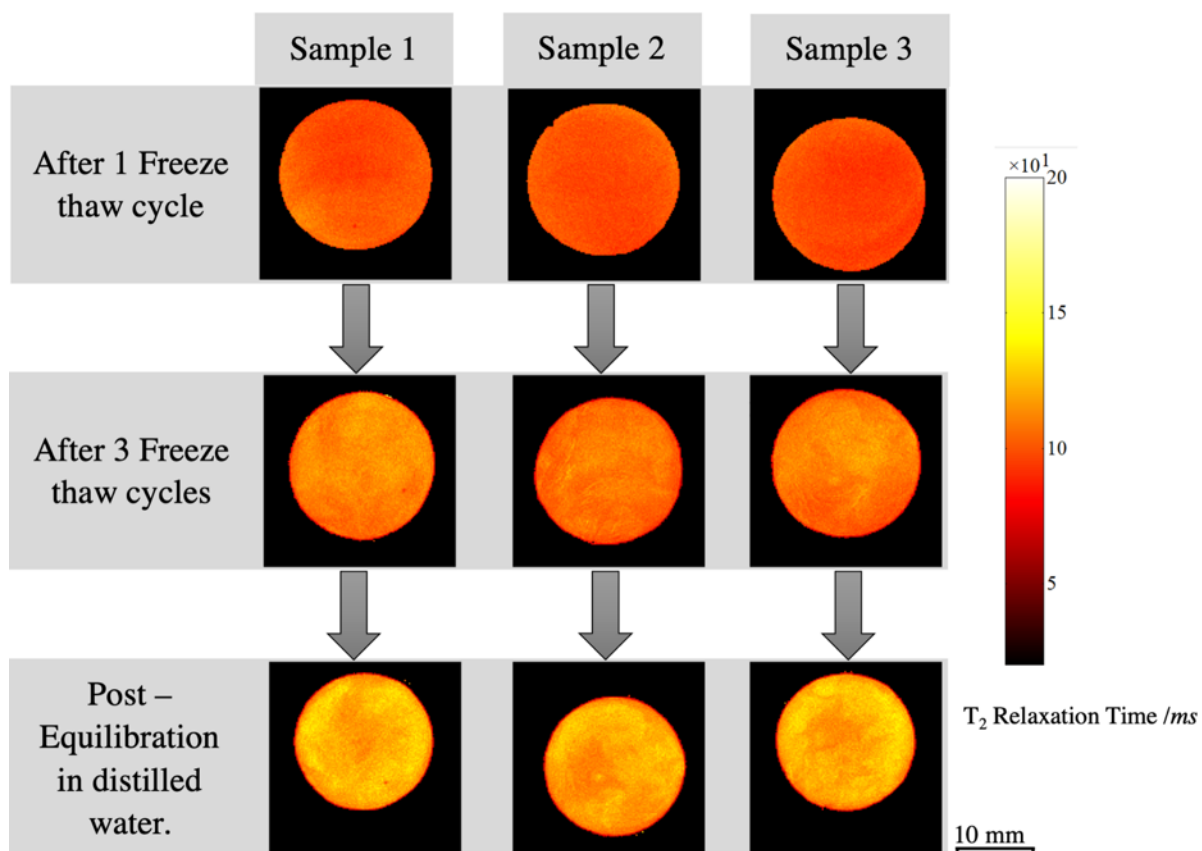


Figure 7: MR T<sub>2</sub> Relaxation maps of three samples of 146-186 kDa PVA with a concentration of 10% w/w, after 1 and 3 cycles, and after 3 days of storage in distilled water to allow for equilibration.

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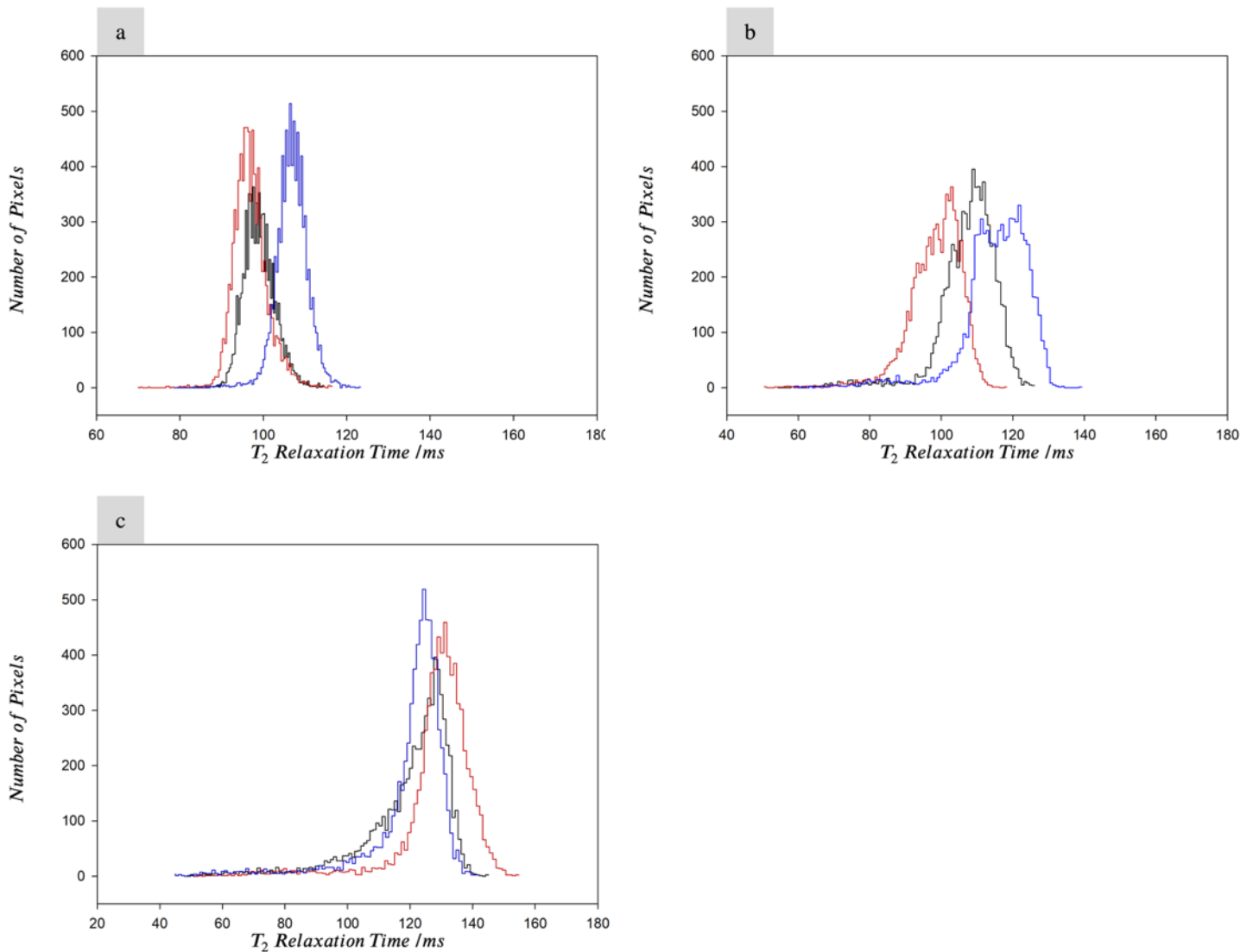


Figure 8: Histograms showing the distribution of MR  $T_2$  relaxation time across all pixels for three samples after 1 (a) and 3 (b) freeze thaw cycle, and after a further 3 days of equilibration (c).

306

307

### 308 **3.3 Viscoelastic properties and MR $T_2$ relaxation time.**

309 Based on the empirical evidence, the PVA-A data could be fitted to an exponential function

310 ( $R^2 = 0.99$ ) between storage modulus and MR  $T_2$  relaxation rate (Figure 9a). For PVA-B a

311 linear relationship ( $R^2 = 0.95$ ) was empirically fitted (Figure 9b). For PVA-C it was not

312 possible to identify a correlation between storage modulus and MR  $T_2$  relaxation rate;

313 therefore, no trend line has been fitted (Figure 9c). These trends (or lack of) were consistent  
 314 for all frequencies.

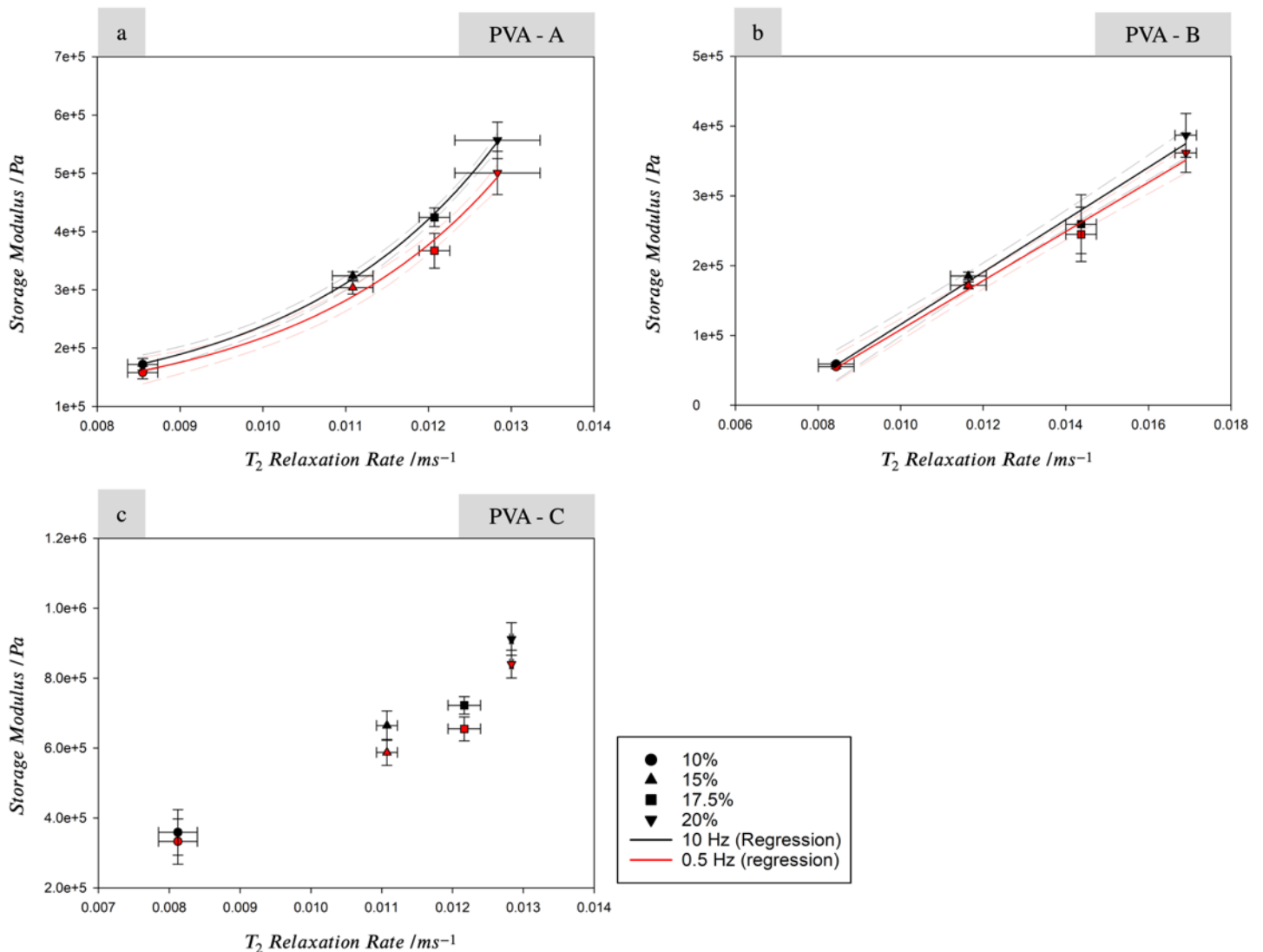


Figure 9: Storage Modulus plotted against MR  $T_2$  Relaxation Rate for 0.5 Hz (red), and 10 Hz (black) for PVA-A (a), PVA-B (b), and PVA-C (c). Error bars show 95% confidence ( $n=6$ ). Regression lines for 0.5 Hz (red), and 10 Hz (black) are also given; dashed lines show 95% confidence intervals for regression.

315 For PVA-A, an exponential empirically-derived relationship was derived between loss  
 316 modulus and MR  $T_2$  relaxation rate ( $R^2 = 0.95$ ) (Figure 10a). It was also noted that the fit  
 317 appeared to improve as frequency decreased, with  $R^2$  ranging from 0.98 at 0.5 Hz to 0.94 at  
 318 10 Hz. A linear relationship was observed between loss modulus and MR  $T_2$  relaxation rate  
 319 for PVA-B ( $R^2 = 0.88$ ) (Figure 10b).  $R^2$  increased from 0.70 at 0.5 Hz to 0.94 at 10 Hz. As  
 320 with the comparison between storage modulus and MR  $T_2$  relaxation rate for PVA-C, it was

321 not possible identify a correlation between loss modulus and MR  $T_2$  Relaxation rate;

322 therefore, no trendline has been presented (Figure 10c).

323

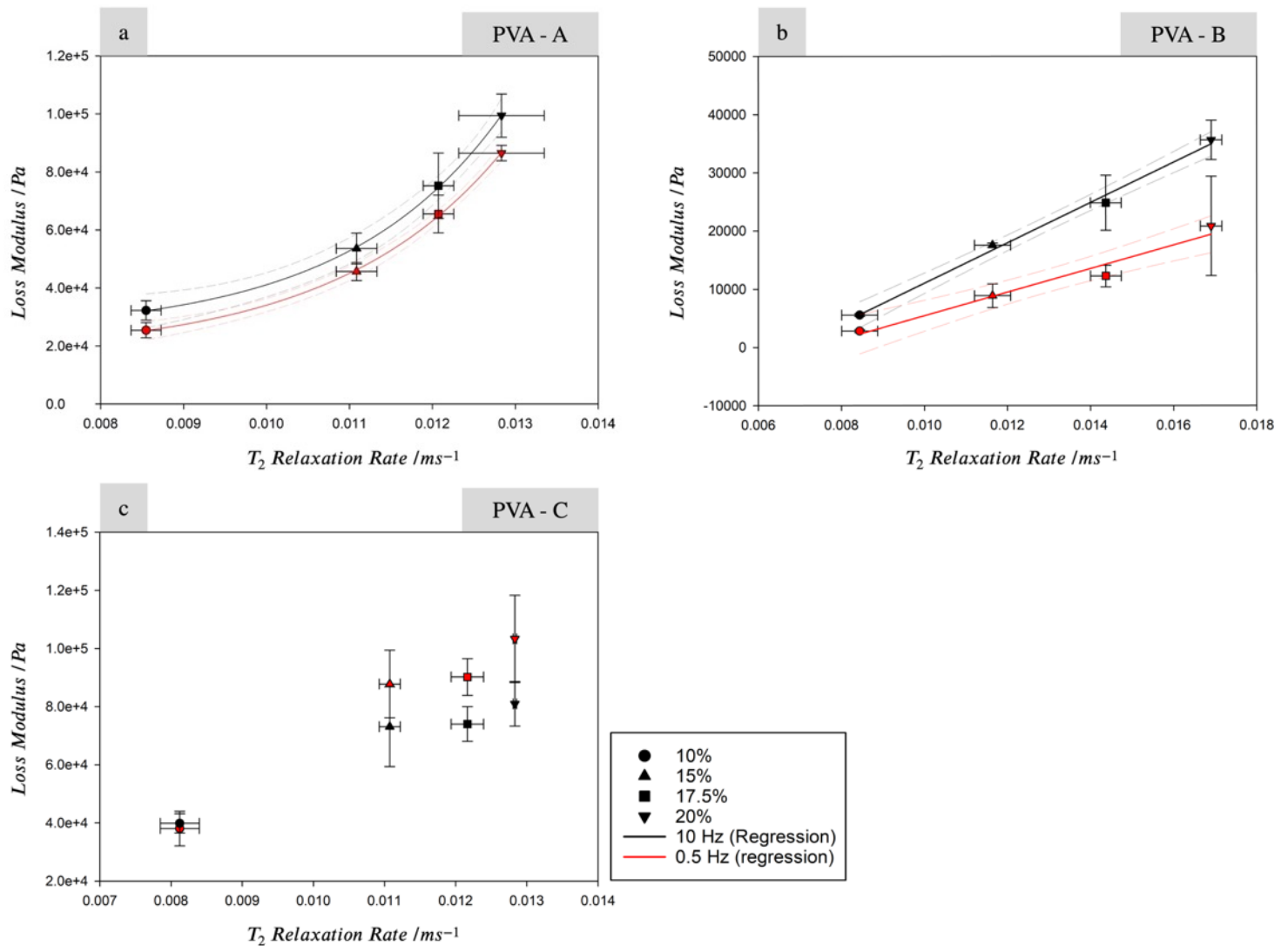


Figure 10: Loss Modulus plotted against MR  $T_2$  Relaxation Rate for 0.5 Hz (red), and 10 Hz (black) for PVA-A (a), PVA-B (b), and PVA-C (c). Error bars show 95% confidence (n= 6). Regression lines for 0.5 Hz (red), and 10 Hz (black) are also given; dashed lines show 95% confidence intervals for regression.

#### 324 **4. Discussion**

325 This study evaluated the effect of PVA concentration for two MWs, 1 and 3 FTCs on the

326 dynamic viscoelastic properties, characterised using DMA, showing the potential to control

327 the viscoelastic mechanical properties of PVA biomaterials by varying polymer composition

328 and number of FTC. This study has also demonstrated the potential to link routine clinical

329 imaging to the material properties of PVA. For PVA-A and B, there was a statistically  
330 significant correlation, between increasing concentration of the polymer and an increase in  
331 the storage and loss moduli. The storage modulus was shown to increase with concentration  
332 for PVA-C, however no statistically significant increase was seen in loss modulus between 15  
333 and 20% ( $p > 0.05$ ). Furthermore, the dynamic (complex) modulus increased with  
334 concentration for all compositions. This increase in the parameters of viscoelasticity as  
335 concentration is increased, is in agreement with previous literature, where a higher  
336 concentration of PVA resulted in a higher Young's Modulus [34]. It was noted that for PVA-  
337 C, that whilst a linear relationship was identified between complex modulus and  
338 concentration, a non-linear relationship was identified between loss modulus and  
339 concentration. As the concentration of PVA-C increases, the behaviour of the loss moduli  
340 implies that the sample is storing a greater proportion of energy, to enable elastic recoil, and  
341 therefore dissipating a lower proportion of energy. This behaviour is only occurring for PVA-  
342 C samples, which are a higher MW composition, and have undergone 3 FTC.

343

344 Previous research, modelling the poro-viscoelastic properties of PVA, showed that it can be  
345 described as a biphasic material [35]. However, in this study, the difference in viscoelastic  
346 response with the proportion of water in the hydrogel, does not correlate with loss modulus  
347 for all compositions i.e. PVA-C. Other studies have shown that as the concentration and MW  
348 of PVA is increased, an increase in polymer cross-linking occurs during FTCs, resulting in a  
349 denser polymer structure and a reduction in pore size [12], further resulting in an increase in  
350 the mechanical stiffness of the hydrogel [34]. This hypothesis is further supported by the  
351 behaviour of the viscoelastic properties presented in this study. An increase in crosslinking  
352 present in PVA-C compared to PVA-B (due to an increase in FTCs) and PVA-A (due to  
353 MW), increases the restriction on water movement in the samples due to an increase in in

354 polymerisation and decrease in pore size. It is hypothesised that this restriction on the water,  
355 reduces the hydrogel's ability to dissipate energy, as its freedom to flow out of the sample  
356 when a force is applied, will be reduced [36]. This theory is in agreement with several studies  
357 of the creep and stress relaxation properties of PVA; which have shown that as polymer  
358 concentration is increased, the response of creep and stress relaxation decreases [12, 35, 37].  
359 This hypothesis would need to be tested on a wider range of MWs and FTCs, to confirm this  
360 theory. However, this study has shown that whilst compositional changes greatly affect the  
361 viscoelastic properties of PVA, the various parameters (for example concentration) do not  
362 affect different compositions in a consistent manner.

363

364 Miramini et al. [25] conducted a literature review to characterise the mechanical properties of  
365 connective tissue; this study yielded an elastic modulus (or equivalent) range of 0.09 – 10  
366 MPa, 0.2 – 8 MPa and, 0.1 – 7 MPa for coronary, aortic, and femoral arteries respectively,  
367 and <1 – 170 MPa for articular cartilage. This range of values for connective tissue is  
368 extremely wide. Burton et al. [24] showed that the storage modulus of coronary arterial tissue  
369 is even higher (14.47 to 25.82 MPa). Yet, Holzapfel et al. [38] calculated the ultimate tensile  
370 stress of coronary arteries to be between 0.4 – 1.4 MPa. The values for the stiffest  
371 composition of PVA cryogel shown in this study, has a storage and loss moduli (0.8 MPa and  
372 0.1 MPa respectively), which fall on the lower side of these reported literature values.

373

374 <sup>1</sup>H MRI is able to map the distribution of hydrogen within a sample [39]. The lifetime of the  
375 MR signal, is dependent on two primary relaxation times, MR  $T_1$  and MR  $T_2$ , which are  
376 sensitive to the chemical and physical environment of protons in a sample. In particular, both  
377 relaxation times are sensitive to molecular mobility and in the case of MR  $T_2$  relaxation, the  
378 relaxation time decreases with decreasing mobility [39, 40]. Therefore, solids have typically



379 lower MR  $T_2$  relaxation times compared to liquids. Additionally, MR  $T_2$  relaxation time is  
380 also sensitive to chemical exchange and internal magnetic field gradients [41], in the presence  
381 of interfaces between materials of different magnetic susceptibilities, which is measured by  
382 MR  $T_2^*$  [39]. In the case of MRI, measured  $T_2$  relaxation times can be sensitive to diffusion,  
383 to an extent dependent on the experimental imaging parameters. In this study, the imaging  
384 parameters were fixed throughout, and thus relative differences and trends can be identified.

385

386 This study evaluated the effect of PVA concentration for two MWs, 1 and 3 FTCs on MR  $T_2$   
387 relaxation time. It was found that MR  $T_2$  relaxation time decreased as concentration was  
388 increased for PVA-A, B and C (Table 2). The reduction in MR  $T_2$  relaxation time, and thus  
389 signal, correlates with the reduction in the %wt. of water in the samples. This result is in  
390 agreement with Orr et al. [30]. The additional parameters assessed in this study, identified  
391 further trends. There was an increase in MR  $T_2$  relaxation time with FTC, which contradicts  
392 the trend reported by Chu and Rutt [29]. Table 2 also identifies that the difference in MR  $T_2$   
393 relaxation time, between 1 and 3 FTCs, increases with the concentration (previously  
394 unreported). Table 3 shows that there was a reduction in cross-sectional area with the FTCs  
395 and subsequent equilibrium process, which further aligns with results from [29], and is  
396 attributed to water being expelled by crystallite growth. The robustness of the observations  
397 between FTCs and MR  $T_2$  relaxation time are inherently limited due to two discrete data  
398 points. Whilst it potentially identifies two novel trends, these require further investigation to  
399 establish correlation and statistical significant. Nevertheless, some observations can be made  
400 about research methodology, and hypotheses can be drawn based on scientific insight.

401

402 MRI was able to qualitatively and quantitatively assess the change in sample heterogeneity  
403 with manufacturing protocol, showing that an increase in FTCs results in an increased pixel

404 distribution of MR  $T_2$  relaxation time throughout a sample (Table 3, Figure 7 and 8). Figure 8  
405 also shows a deviation from normal distribution after equilibration, with a skew to lower  
406 values of MR  $T_2$  relaxation times. In part, this can be attributed to the ‘halo’ of lower signal  
407 intensity around the edge of the samples (Figure 7), which further agrees with the expulsion  
408 of water described by Chu et al. [29], occurring at the boundary of the sample. The literature  
409 reports that increasing the number of FTCs during manufacture, results in compositional  
410 heterogeneity within the hydrogel [42]. This is due to the formation of ice crystals during the  
411 freezing process, resulting in an increase in the concentration of the polymer solution in  
412 surrounding regions and promoting more interactions between the polymer chains, and  
413 between the polymer and ice crystals [43]. Thawing of the ice crystals, results in pockets of  
414 water, which can lead to a microporous structure throughout the hydrogel. Subsequent FTCs  
415 reinforce this process, which results in the formation of polymer rich and polymer poor  
416 regions [42, 44].

417

418 The generation of water pockets, and increased mobility of the water molecules, in the  
419 microstructure of the material is hypothesised to cause the increase in MR  $T_2$  relaxation time  
420 with FTC. It is important to emphasise, that the increase in MR  $T_2$  relaxation time with FTC,  
421 is attributed to an increase in water mobility, as opposed to water concentration, which  
422 through the cross-sectional area results in Table 3 and [29] was assumed to reduce. This  
423 observation is further strengthened by Table 2, which shows (by comparing PVA-B and  
424 PVA-C) that by reducing %wt. water concentration, has less impact than the increase in water  
425 mobility from additional FTCs. To confirm the microstructural changes of the PVA with FTC  
426 requires further investigation. The phase heterogeneity of PVA has been previously measured  
427 using different experimental methods, including X-ray diffraction [45], small angle neutron  
428 scattering [46, 47], and confocal laser scanning microscopy [48]. MRI is not able to resolve

429 the microscopic pore structure of PVA however, this study has demonstrated that the  
430 formation of polymer-rich and polymer-poor regions can be indirectly measured through the  
431 resulting variation in polymer and water content of these regions and thus the signal. It is  
432 unlikely that this microscopic variation in composition has an impact on the viscoelastic  
433 properties of the hydrogels, as these are bulk measurements.

434

435 Comparing Tables 2 and 3, the mean MR  $T_2$  relaxation time for 10% PVA-C in Table 2  
436 (123ms) is equivalent to 10% PVA-C after 3 days of equilibrating in water in Table 3  
437 (122ms). Reflecting on the difference in methodology between these experiments, the  
438 difference in MR  $T_2$  relaxation time pre and post equilibration ( $\sim 10\%$ ), demonstrates the  
439 critical importance of the storage conditions of PVA cryogels. This parameter was not  
440 reported in [29], and was approximately 4 weeks in [30]. Chu and Rutt [29] also described  
441 different manufacturing and imaging protocol, most notably, they state long echo times,  
442 which would increase susceptibility to internal magnetic field gradients. To robustly  
443 determine the source of the opposing trends between MR  $T_2$  relaxation time and FTC reported  
444 in [29] and this study an increased and more detailed assessment of manufacturing  
445 parameters, under matched imaging parameters, would be required.

446

447 Finally and importantly, this paper has demonstrated, for the first time, a correlation between  
448 viscoelastic mechanical properties and the MR  $T_2$  relaxation time for a defined composition.  
449 Note that “correlation does not imply causation”, and in this case both the viscoelastic and  
450 MRI properties of PVA are dependent on the hydrogel’s water content. The mechanical  
451 properties and MR  $T_2$  relaxation time of PVA are correlated because both are affected by  
452 changes in PVA composition. This unique result is exciting because it suggests the possibility  
453 of MRI based quantification of the composition-dependent mechanical properties of PVA.

454 Such a development would have huge ramifications in the development and application of  
455 tissue scaffolds, implants and phantoms. Future development and validation could allow in-  
456 situ and non-invasive imaging of the mechanical function of PVA cryogels, with the wider  
457 principles outlined in this paper also applicable to other biocompatible hydrogels [49].  
458 Magnetic resonance elastography (MRE) is currently the most researched method used to  
459 measure mechanical properties *in vivo*, with PVA being a commonly used phantoms material  
460 to develop MRE [50]. The techniques presented here could be used to enhance and validate  
461 such developments by using *in vivo* imaging to validate mechanical properties and monitor  
462 changes in homogeneity in a sample or implant.

463

464 The advent of more advanced manufacturing techniques for hydrogels, such as additive  
465 manufacture, leads to the ability to create more complex geometries, [18] and multi-material  
466 models [51, 52]. The findings from this study have the potential to aid such developments by  
467 demonstrating how to alter, and control, the mechanical properties of PVA by varying the  
468 composition of the polymer and the parameters of additive manufacturing [53]. These  
469 advances imply the potential for considerably more accurate material models for soft  
470 connective tissues, allowing for changing mechanical properties throughout an implant or  
471 phantom [54]. More specifically, this study demonstrates the feasibility of optimising the  
472 viscoelastic material properties of PVA based on this hydrogel's composition. Therefore,  
473 allowing the results shown in this study to be used in tandem with more complex  
474 manufacturing methods, to further tune the mechanical behaviour of PVA. This information  
475 will also allow researchers to be more informed when selecting materials, such as for medical  
476 devices or imaging phantoms, and has applications to further improve the accuracy of  
477 material models of PVA. For example, the frequency domain data presented in this study is

478 available for the analysis of stress of medical devices which use PVA [9, 55, 56] under  
479 dynamic loading conditions which mimic physiological loading [57].

480

481 This study has successfully shown the impact of the compositional and manufacturing  
482 parameters of PVA cryogel on its viscoelastic properties and MR  $T_2$  relaxation time. Future  
483 research will require an increased number of parameters to explore the impact on the loss  
484 modulus and MR  $T_2$  relaxation time. Further to this, an increased number of FTCs would be  
485 required to increase the dynamic moduli of the cryogel to the equivalent range for some  
486 arterial tissue reported in the literature.

487

#### 488 ***Conclusion***

489 This study has explored the effect of MW, concentration and the manufacturing protocol of  
490 PVA cryogel on its viscoelastic mechanical properties and the MR  $T_2$  Relaxation time. It was  
491 shown that:

- 492 • An increase in concentration results in an increase in storage and loss moduli.
- 493 • The trend between concentration and viscoelastic moduli is dependent on the MW and  
494 the manufacturing protocol.
- 495 • A linear relationship exists between MR  $T_2$  relaxation rate and concentration,  
496 inclusive of both MWs and variations in manufacturing protocol.
- 497 • MRI can qualitatively and quantitatively identify sample heterogeneity, which was  
498 shown to increase with number of FTCs and further equilibrating in water.

499 Further to these results, this research demonstrated a causal relationship between the MR  $T_2$   
500 relaxation rate and viscoelastic properties of PVA. Excitingly, this demonstrates the potential  
501 to image the composition-dependent viscoelastic properties of PVA remotely. Such an attribute

502 could enable future in-situ and non-invasive identification of the mechanical properties of  
503 implants, tissue scaffolds and phantoms.

504

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506

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510

## 511 **7. Conflict of Interest**

512 The authors declare that there is no conflict of interest.

513

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## 520 **8. References**

521

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680 **Supplementary data:**681 *Table S1: Storage Modulus (Mean  $\pm$  SD) for all PVA-A samples tested at frequencies between 0.5-10 Hz.*

Freq. (Hz)	Storage Modulus (Pa)							
	10% W/W		15% W/W		17.5% W/W		20% W/W	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	158000	9500	298000	5500	362000	16000	484000	24000
1	159000	8400	301000	3900	373000	15000	501000	22000
2	163000	8500	308000	4700	388000	14000	518000	24000
3	165000	8600	312000	5200	397000	13000	529000	25000
4	167000	8600	315000	5400	404000	13000	535000	26000
6	169000	8700	319000	5800	414000	13000	546000	26000
8	171000	8800	323000	5900	421000	14000	552000	27000
10	172000	8800	324000	5900	425000	14000	557000	27000

682 *Table S2: Storage Modulus (Mean  $\pm$  SD) for all PVA-B samples tested at frequencies between 0.5-10 Hz.*

Freq. (Hz)	Storage Modulus (Pa)							
	10% W/W		15% W/W		17.5% W/W		20% W/W	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	55000	2400	172000	4400	245000	36000	361000	25000
1	55700	2200	175000	4800	247000	36000	367000	27000
2	56600	2200	177000	4900	251000	37000	373000	27000
3	57100	2200	179000	5000	253000	37000	376000	28000
4	57500	2300	180000	5000	254000	37000	378000	28000
6	58100	2300	183000	5100	256000	38000	382000	28000
8	58500	2300	184000	5200	258000	38000	385000	28000
10	58900	2300	185000	5200	259000	39000	387000	29000

684 *Table S3: Storage Modulus (Mean  $\pm$  SD) for all PVA-C samples tested at frequencies between 0.5-10 Hz.*

Freq. (Hz)	Storage Modulus (Pa)							
	10% W/W		15% W/W		17.5% W/W		20% W/W	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	333000	59000	588000	34000	655000	31000	840000	36000
1	336000	59000	605000	35000	674000	30000	862000	39000
2	343000	60000	627000	37000	692000	28000	883000	40000
3	348000	60000	639000	37000	702000	27000	892000	41000
4	350000	60000	646000	38000	708000	26000	898000	41000
6	355000	60000	656000	38000	715000	25000	906000	42000
8	357000	60000	661000	38000	720000	24000	909000	42000
10	359000	59000	664000	38000	722000	23000	912000	43000

686 *Table S4: Loss Modulus (Mean  $\pm$  SD) for all PVA-A samples tested at frequencies between 0.5-10 Hz.*

Freq. (Hz)	Loss Modulus (Pa)			
	10% W/W	15% W/W	17.5% W/W	20% W/W

	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	25400	2300	45700	2700	65500	5700	86500	2300
1	26400	2100	46600	2700	67600	6400	88200	3800
2	27200	2400	47400	2900	69200	7900	89300	5100
3	28000	2400	47900	3400	70300	8900	90200	5500
4	28700	2400	48700	3800	70800	9400	91200	5600
6	29900	2700	50100	4200	71800	9800	93500	5900
8	31200	2800	51800	4600	72900	9800	96200	6200
10	32300	2900	53600	4600	75300	9800	99400	6500

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Table S5: Loss Modulus (Mean  $\pm$  SD) for all PVA-B samples tested at frequencies between 0.5-10 Hz.

Freq. (Hz)	Loss Modulus (Pa)							
	10% W/W		15% W/W		17.5% W/W		20% W/W	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	2820	240	8890	1800	12300	1700	20900	7800
1	2980	200	8920	890	12800	1900	19700	5100
2	3260	160	9670	550	13800	2200	20400	3500
3	3550	150	10700	420	15200	2500	22100	2900
4	3820	150	11700	370	16600	2800	24000	2800
6	4410	170	13700	300	19400	3300	27900	2700
8	4990	180	15600	310	22200	3900	31700	2900
10	5550	210	17600	340	24800	4300	35700	3100

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Table S6: Loss Modulus (Mean  $\pm$  SD) for all PVA-C samples tested at frequencies between 0.5-10 Hz.

Freq. (Hz)	Loss Modulus (Pa)							
	10% W/W		15% W/W		17.5% W/W		20% W/W	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	38100	5400	87800	11000	90200	5800	103000	14000
1	38700	4900	87100	13000	85300	6000	95000	10000
2	37900	3900	81900	15000	78400	7000	84700	10000
3	37400	3400	77800	15000	74800	7100	79700	9500
4	37300	3100	75000	15000	72600	7000	77200	9000
6	37700	2700	72300	14000	71300	6500	76100	8000
8	38600	2800	71900	13000	72000	5900	77800	7400
10	39900	3000	73100	13000	74000	5500	81000	7000

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693 *Table S7: Storage and Loss constants with respect to concentration of PVA-A and PVA-B for frequencies*  
 694 *between 0.5 and 10 Hz. (Equations 7 & 8)*

	Freq. (Hz)	Storage Modulus (Pa)			$R^2$	Loss Modulus (Pa)			
		$a_s$	$b_s$	$d_s$		$a_l$	$b_l$	$d_l$	$R^2$
PVA-A	0.5	-86400	108000	0.083	0.98	-1640	8080	0.12	0.97
	1	-70600	94800	0.09	0.98	-2170	8690	0.117	0.97
	2	-75700	97400	0.09	0.98	-2780	9390	0.115	0.95
	3	-76400	98000	0.091	0.99	-1630	9140	0.116	0.94
	4	-86700	104000	0.089	0.99	157	8550	0.119	0.94
	6	-99200	113000	0.087	0.99	3480	7460	0.125	0.93
	8	-108000	118000	0.086	0.99	6680	6470	0.132	0.93
	10	-103000	115000	0.087	0.99	7360	6510	0.133	0.94
PVA-B	0.5	-92000	48700	0.114	0.96	-339	536	0.183	0.94
	1	-89000	46800	0.114	0.96	-2320	1330	0.14	0.83
	2	-88700	46800	0.114	0.96	-4360	2390	0.117	0.9
	3	-91600	48000	0.114	0.96	-5950	3260	0.107	0.92
	4	-91600	48000	0.114	0.96	-7560	4160	0.101	0.93
	6	-90700	47900	0.114	0.96	-9720	5360	0.0973	0.94
	8	-91600	48500	0.114	0.96	-12000	6640	0.0941	0.94
	10	-90300	47600	0.112	0.96	-13600	7500	0.0939	0.93

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*Table S8: Storage constants with respect to concentration of PVA-C for frequencies between 0.5 and 10 Hz. (Equation 9)*

	Freq. (Hz)	$f$	$g$	$R^2$
PVA-C	0.5	-167000	53200	0.93
	1	-171000	50600	0.94
	2	-174000	51900	0.94
	3	-174000	52400	0.94
	4	-173000	52700	0.94
	6	-170000	53000	0.94
	8	-168000	53100	0.94
	10	-158000	48700	0.93

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*Table S9: Mean MR  $T_2$  relaxation rate constants for PVA-A, B, and C. (Equation 10)*

	$x$	$y$	$R^2$
PVA-A	0.0040	0.00043	0.99
PVA-B	-0.00020	0.00080	0.99
PVA-C	0.0034	0.00050	0.97

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703 *Table S10: Multiplication factors comparing variation of viscoelastic properties when MW is increased from*  
 704 *89-98 kDa to 146-186 kDa (10-20% PVA-A and C) between frequencies of 0.5 and 10 Hz.*

Freq. (Hz)	Storage Modulus (Pa)				Loss Modulus (Pa)			
	10% W/W	15% W/W	17.5% W/W	20% W/W	10% W/W	15% W/W	17.5% W/W	20% W/W
0.5	2.1 ×	2.0 ×	1.8 ×	1.7 ×	1.5 ×	2.0 ×	1.4 ×	1.2 ×
1	2.1 ×	2.0 ×	1.8 ×	1.7 ×	1.5 ×	1.9 ×	1.3 ×	1.1 ×
2	2.1 ×	2.0 ×	1.8 ×	1.7 ×	1.4 ×	1.7 ×	1.1 ×	0.93 ×
3	2.1 ×	2.1 ×	1.8 ×	1.7 ×	1.3 ×	1.6 ×	1.1 ×	0.87 ×
4	2.1 ×	2.1 ×	1.7 ×	1.7 ×	1.3 ×	1.5 ×	1.0 ×	0.83 ×
6	2.1 ×	2.1 ×	1.7 ×	1.7 ×	1.3 ×	1.4 ×	1.0 ×	0.80 ×
8	2.1 ×	2.1 ×	1.7 ×	1.7 ×	1.2 ×	1.4 ×	1.0 ×	0.80 ×
10	2.1 ×	2.1 ×	1.7 ×	1.6 ×	1.2 ×	1.4 ×	1.0 ×	0.80 ×
<b>Average</b>	<b>2.1 ×</b>	<b>2.0 ×</b>	<b>1.8 ×</b>	<b>1.7 ×</b>	<b>1.2 ×</b>	<b>1.6 ×</b>	<b>1.1 ×</b>	<b>0.91 ×</b>
<b>SD</b>	<b>0.0083</b>	<b>0.027</b>	<b>0.039</b>	<b>0.033</b>	<b>0.094</b>	<b>0.21</b>	<b>0.14</b>	<b>0.14</b>

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709 *Table S11: Multiplication factors comparing variation of viscoelastic properties when the number of freeze*  
 710 *thaw cycles is increased from 1 to 3 (10-20% PVA-B and C) between frequencies of 0.5 and 10 Hz.*

Freq. (Hz)	Storage Modulus (Pa)				Loss Modulus (Pa)			
	10% W/W	15% W/W	17.5% W/W	20% W/W	10% W/W	15% W/W	17.5% W/W	20% W/W
0.5	6.1 ×	3.4 ×	2.7 ×	2.3 ×	14 ×	9.9 ×	7.3 ×	4.9 ×
1	6.0 ×	3.5 ×	2.7 ×	2.3 ×	13 ×	9.8 ×	6.7 ×	4.8 ×
2	6.1 ×	3.5 ×	2.8 ×	2.4 ×	12 ×	8.5 ×	5.7 ×	4.2 ×
3	6.1 ×	3.6 ×	2.8 ×	2.4 ×	11 ×	7.3 ×	4.9 ×	3.6 ×
4	6.1 ×	3.6 ×	2.8 ×	2.4 ×	9.8 ×	6.4 ×	4.4 ×	3.2 ×
6	6.1 ×	3.6 ×	2.8 ×	2.4 ×	8.5 ×	5.3 ×	3.7 ×	2.7 ×
8	6.1 ×	3.6 ×	2.8 ×	2.4 ×	7.7 ×	4.6 ×	3.2 ×	2.5 ×
10	6.1 ×	3.6 ×	2.8 ×	2.4 ×	7.2 ×	4.2 ×	3.0 ×	2.3 ×
<b>Average</b>	<b>6.1 ×</b>	<b>3.5 ×</b>	<b>2.8 ×</b>	<b>2.4 ×</b>	<b>10 ×</b>	<b>7.0 ×</b>	<b>4.9 ×</b>	<b>3.5 ×</b>
<b>SD</b>	<b>0.026</b>	<b>0.063</b>	<b>0.039</b>	<b>0.015</b>	<b>2.2</b>	<b>2.1</b>	<b>1.5</b>	<b>0.97</b>

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712

713 Table S12: Storage and loss constants with respect to MR  $T_2$  relaxation rate of PVA-A for frequencies between  
 714 0.5 and 10 Hz. (Equations 11 & 13)

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Freq. (Hz)	Storage Modulus (Pa)				Loss Modulus (Pa)			
	$a_s$	$b_s$	$d_s$	$R^2$	$a_l$	$b_l$	$d_l$	$R^2$
0.5	86800	2680	388	0.98	17000	119	496	0.98
1	91100	2200	406	0.98	17500	130	491	0.97
2	91900	2210	409	0.98	17900	143	485	0.95
3	93400	2180	412	0.98	18800	137	488	0.94
4	91900	2340	408	0.99	19800	124	496	0.94
6	90600	2530	404	0.99	21600	102	511	0.93
8	89600	2670	401	0.99	23400	83.2	528	0.93
10	92000	2560	405	0.99	24400	82.8	531	0.94

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718 Table S13: Storage and loss constants with respect to MR  $T_2$  relaxation rate of PVA-B for frequencies between  
 719 0.5 and 10 Hz. (Equations 12 and 14)

Freq. (Hz)	Storage Modulus (Pa)			Loss Modulus (Pa)		
	$a_s$	$b_s$	$R^2$	$a_l$	$b_l$	$R^2$
0.5	-243000	35200000	0.95	-14900	2030000	0.70
1	-247000	35700000	0.95	-13500	1910000	0.82
2	-250000	36200000	0.95	-13500	1970000	0.89
3	-252000	36500000	0.95	-14500	2130000	0.92
4	-254000	36700000	0.95	-15700	2320000	0.92
6	-256000	37000000	0.95	-18400	2700000	0.93
8	-257000	37300000	0.95	-20900	3080000	0.94
10	-259000	37500000	0.95	-23500	3460000	0.94

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