INTRODUCTION
Rupture of the anterior cruciate ligament (ACL) has been estimated to occur at an annual rate of 1 in 3000 in the US alone, translating to over 100,000 reconstruction surgeries to restore joint stability [1].

The decellularisation of xenogenic tissues may offer a promising alternative to direct autologous or allogeneic solutions by delivering immunologically safe reconstructive biomaterials in plentiful supply. However, it is necessary to quantify the effects of the decellularisation process and sterilisation methods on the biomechanics of the proposed grafts, particularly their viscoelasticity since they are intended to encounter repetitive loading conditions.

DECELLULARISATION
Super flexor tendons were harvested from 4-6 month old large white pigs. Decellularisation was achieved using an adaption of a previously used protocol for the meniscus [2].

For this study, a bioburden reduction step was introduced early in the process using either peracetic acid (PAA; 0.1% w/v) or an antibiotic wash and a terminal sterilisation step incorporated late in the process using PAA (0.1% w/v).

In total 6 decellularised groups (each at n=6) were analysed; decellularised specimens with & without terminal PAA treatment, decellularised specimens with & without terminal PAA treatment in addition to a PAA bioburden reduction step and decellularised specimens with & without terminal PAA treatment in addition to an antibiotic bioburden reduction step.

A fresh control group (n=6) was also included for the purposes of comparison.

TEST SET-UP
Specimens were processed into ‘dumbbell’ shapes of consistent dimensions before being subjected to stress relaxation testing using bespoke cryo-grips in series with an Instron 3365 materials testing system.

CHARACTERISATION
Testing comprised of a ramp displacement phase at 30mm/min until a stress of 5MPa was achieved. At this point the strain (ε_0) remained fixed for a period of 5mins while stress relaxation (σ(t)) was recorded. The stress relaxation modulus (E(t)/σ(0)/ε_0) was calculated and fitted to a modified Maxwell-Wieruch model [3]:

\[ E(t) = E_1 + \frac{1}{L_2} \sum_{i=1}^{n} E_{1i} \tau_i e^{-\tau_i t} (e^{\tau_i t} - 1) \]

The simplest form of the model consists of two Maxwell elements in parallel with a single spring (i.e. n = 2). \( E_1 \) is the time-independent elastic modulus of the single spring, whereas \( E_{1i} \) and \( \tau_i \) represent the time-dependent elastic modulus and relaxation time respectively of the Maxwell elements and \( \tau_0 \) is the ramp time.

RESULTS
The best-fit viscoelastic parameters for all groups are presented in Table 1. For all elastic moduli \( (E_1, E_{01}, E_{02}) \), a significant difference was found between all decellularised groups and the fresh control. Terminal PAA treatment reduced \( E_0 \) of standard decellularised specimens. The introduction of PAA as a bioburden reduction step had an additional significant negative effect on \( E_0 \), reducing it further with or without terminal PAA treatment.

DISCUSSION
The reduction of the baseline elasticity \( (E_0) \) can be attributed to an increase in extensibility due to un-crimping of the tendon collagen fibers. The viscosity of each Maxwell element in the model applied can be calculated by the product of its time-

\[ \eta_i = \tau_i \times E_{1i} \]

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addition steps investigated.

Table 1. Viscoelastic parameters of the modified Maxwell-Wieruch model. Fresh = fresh control, D’cell = decellularised, PAA-bio = peracetic acid bioburden reduction, Anti-bio = antibiotic bioburden reduction. Results shown as mean ± 95% CI (n=6 in all cases). Superscripts indicate significance – groups that do not share the same letter are significantly different (1-way ANOVA with Tukey post-hoc).

<table>
<thead>
<tr>
<th>Group</th>
<th>( E_1 ) (MPa)</th>
<th>( E_0 ) (MPa)</th>
<th>( \eta_0 ) (Pa·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>49.67 ± 4.92</td>
<td>6.97 ± 6.72</td>
<td>7.37 ± 6.97</td>
</tr>
<tr>
<td>D’cell</td>
<td>49.67 ± 4.92</td>
<td>6.97 ± 6.72</td>
<td>7.37 ± 6.97</td>
</tr>
<tr>
<td>D’cell +PAA</td>
<td>49.67 ± 4.92</td>
<td>6.97 ± 6.72</td>
<td>7.37 ± 6.97</td>
</tr>
<tr>
<td>D’cell +Anti-bio</td>
<td>49.67 ± 4.92</td>
<td>6.97 ± 6.72</td>
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</tbody>
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Financial Disclosure
1. J. Fisher is a paid consultant to DePuy Synthes Joint Reconstruction, Invibio, TissueRegenics Group plc and a share holder of Tissue Regenics Group plcc.
2. E. Ingham is a paid consultant to DePuy Synthes Joint Reconstruction, Stryker, Tissue Regenics Group plc and a share holder of Tissue Regenics Group plcc.