RELAXATION AND RECOVERY OF COLON AFTER APPLICATION OF A MECHANICAL STRESS

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ABSTRACT
Introduction
The colon is a soft biological tissue that exhibits complex viscoelastic behaviour. The aim of this study is to mechanically model relaxation of colonic tissue after indentation, to eventually quantify tissue recovery after stress application.

Methods
A single stress value of 51kPa was applied to ex vivo porcine colon for 5, 30 and 60 seconds. 20 indentations were applied to either the mucosal or muscular layers. The relaxation portion was analysed by calculating the difference between the highest and lowest force (ΔF). The Maxwell and Wiechert models were fitted to the data and compared. Histological evidence of tissue damage was assessed.

Results
Mean ΔF was higher with indentations at the muscular surface as compared to mucosal surface at 5 and 30s time-points. This reversed at 60s. The Wiechert model fit improved with longer duration (30 and 60s). Evidence of histological damage was found consistently in the stressed region.

Conclusion
The Maxwell model is the simplest form that exhibits viscoelastic behaviour, but refining this and linking it to the anatomical tissue structure, using the Wiechert model, provides a better fit. We will now use this model in further studies correlating the model coefficients with histological damage grading.

KEY WORDS
Biomaterials, Biomechanics, Viscoelasticity, Colonic tissue

1. Introduction
The advent of minimally invasive Surgery (MIS) has revolutionised abdominal surgery in the last three decades. In laparoscopic surgery the instruments are inserted through 10-20mm “key hole” incisions. Laparoscopic surgery relies on tissue manipulation by surgical instruments. Current laparoscopic graspers provide the surgeon with little or no haptic feedback, leading to the potential for application of inappropriate forces at the instrument-tissue interface. If grasping forces are too low contact with tissue is lost, but excessive force can lead to irreversible damage. Most graspers comprise two hinged jaws with teeth for tissue traction and blunt metal tips. In laparoscopic colorectal surgery there is a risk of damage to the colon and surrounding structures from these instruments. Most traumatic injuries are likely to be subclinical, not affecting the patient’s outcome. However a 3.6% mortality rate has been related to grasper induced intestinal injury [1]. There has been little scientific investigation of the nature of instrument induced stresses involved in laparoscopic surgery and their impact on tissues. The most notable attempt is contained in the thesis by De [2]. Using an in vivo porcine model, representative stresses were applied to liver, ureter and small bowel using a motorized endoscopic grasper. Histological indicators of tissue damage, including cell death and inflammation, were measured. A graded, non-linear response between applied stress and histological markers of damage and inflammation were observed in liver and small bowel [3].

The colon is a soft biological tissue that exhibits complex mechanical characteristics including viscoelastic, non-linear, anisotropic and inhomogeneous behaviour. Viscoelasticity is the product of fluid flow resistance (viscosity) and solid behaviour (elasticity) within the cellular level of soft tissues. The viscoelastic properties of soft tissue can be explained by spring-damper physics-based models, where the spring represents the elastic solid-like and dashpot exhibits viscous fluid-like behaviour. Many approaches have been used to model the time-dependent response of solid soft tissues, including the widely used and well accepted quasilinear viscoelastic theory by Fung [4]. Investigating the mechanical properties of hollow organs is more complex because parameters such as wall thickness can vary and materials contained within the organ (e.g. gases in the intestines) will have a significant effect on the compound properties of the organ.

Two fundamental approaches exist for developing models of soft tissue mechanical behavior; constitutive, physical law-based models and phenomenological models based on curve-fitting experimental data. Constitutive models lead to easier extraction of the physical meaning of the
parameters but may not have perfect fits with acquired data. Phenomenological models have little or no physical relevance but may achieve excellent fits to the acquired data with potentially less computationally intensive functions [5].

The aim of this study is to develop and evaluate a methodology to mechanically model the relaxation of colonic tissue after indentation. This is part of a broader aim to quantify tissue recovery, and ultimately the extent of irreversible damage, after the application of a mechanical stress and correlate this with histological measures of tissue damage.

2. Methods

2.1 Tissue indentation

Mechanical stresses were applied to fresh, ex vivo porcine colon using a modular universal surface tester (MUST tester). This is an indentation device that applies load to tissue and monitors the resultant penetration depth of the indenter. Force measurement is achieved by employing a parallel spring set in contact with the indenter. A micro mirror is attached to the tip of each spring. As the indenter presses on the material, the springs and so the mirrors are deflected. A fibre optic sensor, placed on the cantilever unit, detects the motion of the springs by emitting light to the mirrors and capturing the reflection. The reflected light is then converted to an electrical signal representing the distance between the deflected spring and the optical sensor. The embedded data acquisition and control unit is calibrated to measure the spring displacement and translate it into a force measurement. The output data are formed into the force against displacement curve and force-time graphs are formed, as shown in Figure 1.

![Figure 1: Force-time graph showing relaxation and how this corresponds to tissue indentation](image)

2.2 Mechanical analysis

Force-time curves from each indentation were analysed to isolate the relaxation portion of the data. The difference between the highest force and the lowest force over the relaxation period (denoted \( \Delta F \)) is calculated (Figure 2). This parameter gives a quantitative measure of the level of irreversible damage to the tissue. For each time variable the \( \Delta F \) for the muscle and mucosal indentations is compared.

![Figure 2: Force-time curve showing calculation of \( \Delta F \)](image)

2.3 Data modelling

To investigate the recovery of porcine colon after indentation further, a least squares method in MATLAB was used to fit parametric models to the experimental data. Two models were considered; the Maxwell model (a basic viscoelastic model) and the Wiechert Model...
consisting of two parallel Maxwell models in parallel with a spring. This approach was adopted to enable the model elements to be related to the physical structure and constitution of the tissue. The Maxwell solid is a mechanical model in which a Hookean spring and a Newtonian dashpot are connected in series. In the series connection the stress on each element is the same and equal to the imposed stress, while the total strain is the sum of the strain in each element [6]. Figure 3 depicts this.

![Maxwell model diagram](image)

**Figure 3:** The Maxwell model of a spring and dashpot in series

The Maxwell model was chosen as it is basic model for viscoelasticity [6]. The limitation of this model is that it can only be used in instances where the stress decays to zero and it is unable to model soft tissues with non-linear elasticity. From this we see that a particular combination of springs and dashpots need to be used to model the mechanical characteristics of tissue. A combination of two parallel Maxwell bodies in parallel with a spring, also known as Wiechert model [6], was then investigated. A Wiechert model can have as many springs and dashpots added to the model as required to correspond to the physical properties of tissue. The structure of colonic tissue is that of a hollow viscous with a double layer of muscle on the outside known as the serosa. This consists of an inner circular and outer longitudinal muscle layer. From outside to inside is the longitudinal muscle, circular muscle, submucosa, muscularis mucosa and mucosa. The equation governing the Wiechert time dependent relaxation modulus $E(t)$ (or tensile stress with time) is shown in Eq 2:

$$E(t) = E_1 + \sum_{i=2}^{n} E_i e^{-t/\tau_i} = E_1 + E_2 e^{-t/\tau_2} + E_3 e^{-t/\tau_3}$$

(2)

Where $E_i$ is the spring in parallel, $\tau = n_i/\eta_i$ and ‘n’ is the number of parallel Maxwell units.

Physically, the parallel Maxwell models may represent the thick, muscular serosa (1 and 2 corresponding to longitudinal and circular muscle), with the parallel spring representing the submucosa (3), muscularis (4) and mucosal layers (5), as shown in Figure 4.

![Wiechert model diagram](image)

**Figure 4:** Wiechert model showing physical representations of colonic tissue

### 2.4 Histological analysis

Haematoxylin and eosin staining was performed to analyse the tissue’s microscopic architecture and show evidence of physical tissue damage. The thickness of each indented histological layer (identified by India ink staining) was compared to an adjacent control region. Three thickness measurements were taken for the following layers; longitudinal muscle, circular muscle, submucosa and muscularis mucosa. The mucosa was graded according to damage to the delicate crypts which make up this layer. Grading of mucosal integrity over the indented area goes from 0 to III as follows:

- 0= Intact mucosa
- I= Under 50% of crypts disrupted
- II=Over 50% of crypts disrupted
- III=Absent crypts down to muscularis mucosa

### 3. Results

#### 3.1 $\Delta F$ Values

Mean and standard deviation $\Delta F$ values are shown for each experimental variable in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (mN)</th>
<th>SD (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5s Mucosa</td>
<td>164.3</td>
<td>40.9</td>
</tr>
<tr>
<td>5s Muscle</td>
<td>197.3</td>
<td>29</td>
</tr>
<tr>
<td>30s Mucosa</td>
<td>306.6</td>
<td>48.4</td>
</tr>
<tr>
<td>30s Muscle</td>
<td>401</td>
<td>51</td>
</tr>
<tr>
<td>60s Mucosa</td>
<td>528.2</td>
<td>83.2</td>
</tr>
<tr>
<td>60s Muscle</td>
<td>335.1</td>
<td>58.5</td>
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</table>
The mean ΔF value was higher with indentations at the serosal, or muscular, surface as compared to mucosal surface at 5 and 30s time-points. This reversed at 60s where the ΔF value was higher at the mucosal surface. Figure 5 shows a box and whisker plot of the ΔF values for each variable. Mean ΔF values at the 60s muscle indentations were lower than that of the 60s mucosa and 30s muscle findings.

Figure 5: Box and whisker plot showing distribution of ΔF values with outliers

### 3.2 Curve fitting data

The Maxwell and Wiechert models were fitted to the measured data for each experimental condition. Mean and standard deviation of the residuals were calculated and are shown in Table 2. The Maxwell model was a better fit that the Wiechert model at 5 second indentations for both mucosa (mean 11.8 Vs 16.94) and muscle (mean 18.4 Vs 34) indentations. For 30 and 60 second indentations the Wiechert model was a consistently better fit on both mucosa and muscle indentations. In the Wiechert model the mean residual was lower for muscle indentations as compared to mucosal at both 30s (5.9 Vs 7.1) and 60s (4 Vs 2) indentations. The fit improved with longer durations for both muscle and mucosal indentations with the Wiechert model.

### 3.3 Histology

All samples in the 30s mucosa condition were not able to be analysed due to disruption of the tissue during processing. Within each testing condition the control and indented sections were compared in terms of thickness of the longitudinal muscle, circular muscle, submucosa and muscularis mucosa. Mean widths for the 60 second muscle indentation are shown in Table 3 and plotted in Figure 6.

Figure 6: Box and whisker plot showing thickness of each histological layer for the 60s muscle indents

### 4. Conclusion

Colon relaxation has been analysed using standard viscoelastic models. The Maxwell model is the simplest form that exhibits the viscoelastic behaviour, but refining this model and linking this to the anatomical tissue structure provides a better fit. The Wiechert model appears to, at longer durations, have a closer fit to tissue relaxation. A testing methodology has been developed which can quantify the level of stress applied to tissue. Supported by histology, the physical, microscopic damage induced can be determined. This opens up the opportunity to systematically link damage to stress for the first time. Microscopic colon damage has been found at a stress level of 51kPa by histology. Muscle recovery appears to reduce with increasing stress duration. We will now use this model in further studies and look to correlate the model coefficients with histological grading of tissue damage.
Table 2: Mean and SD of residuals for the Maxwell and Wiechert models

<table>
<thead>
<tr>
<th></th>
<th>5s Mucosa</th>
<th>5s Muscle</th>
<th>30s Mucosa</th>
<th>30s Muscle</th>
<th>60s Mucosa</th>
<th>60s Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Maxwell</td>
<td>11.8</td>
<td>18.4</td>
<td>12.2</td>
<td>17</td>
<td>18.2</td>
<td>10.3</td>
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<tr>
<td>SD Maxwell</td>
<td>2.91</td>
<td>16.5</td>
<td>1.9</td>
<td>3</td>
<td>3</td>
<td>1.9</td>
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<tr>
<td>Mean Wiechert</td>
<td>16.94</td>
<td>34</td>
<td>7.1</td>
<td>5.9</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>SD Wiechert</td>
<td>8.2</td>
<td>72.4</td>
<td>3.95</td>
<td>4.3</td>
<td>1.5</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 3: Thickness of all layers of control and indented regions for the 60s muscle indentations

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal (µm)</th>
<th>Circular (µm)</th>
<th>Submucosa (µm)</th>
<th>Muscularis (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5s Mucosa Control</td>
<td>132.94</td>
<td>218</td>
<td>596</td>
<td>37</td>
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<tr>
<td>Indent</td>
<td>109.32</td>
<td>196</td>
<td>503</td>
<td>32</td>
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<tr>
<td>5s Muscle Control</td>
<td>251</td>
<td>432</td>
<td>456</td>
<td>44.9</td>
</tr>
<tr>
<td>Indent</td>
<td>185</td>
<td>422</td>
<td>473</td>
<td>39.3</td>
</tr>
<tr>
<td>30s Muscle Control</td>
<td>170.9</td>
<td>412</td>
<td>546.1</td>
<td>49.4</td>
</tr>
<tr>
<td>Indent</td>
<td>133.8</td>
<td>361.5</td>
<td>307.8</td>
<td>43.6</td>
</tr>
<tr>
<td>60s mucosa Control</td>
<td>243</td>
<td>489.8</td>
<td>582.5</td>
<td>47.8</td>
</tr>
<tr>
<td>Indent</td>
<td>228</td>
<td>446.4</td>
<td>430.5</td>
<td>38.3</td>
</tr>
<tr>
<td>60s muscle Control</td>
<td>162.8</td>
<td>286.5</td>
<td>561.7</td>
<td>47.5</td>
</tr>
<tr>
<td>Indent</td>
<td>121.8</td>
<td>253</td>
<td>345.1</td>
<td>41.9</td>
</tr>
</tbody>
</table>

References