Novel In Situ Imaging Approaches to Study Biomechanics

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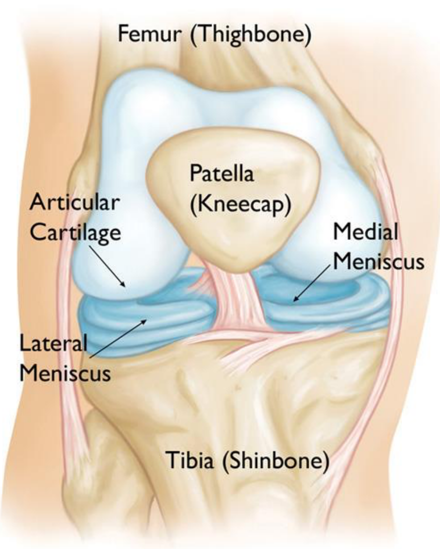
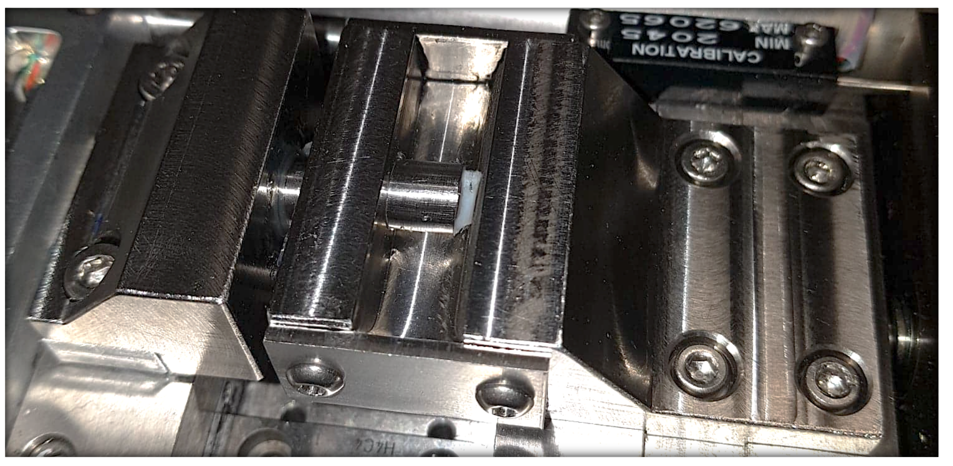


Figure 1. schematic showing location of the two menisci within the knee joint[1]

The Problem: For decades, research has strived for a solution to poor natural regeneration of certain tissues in the body. Biological tissue engineering is advancing quickly with modern technologies, but substantial knowledge about the structure-function relationship of the tissue is required in order for a produced artificial replacement to be effective. One of these poorly healing tissues is the meniscus of the knee, a replacement for which is extremely sought after given its prominent role in joint stability.

Project Aims: **1)** Produce novel data on the meniscus' mechanical behaviour **2)** Inform future research on how modern technologies can be used in the investigation of biological tissues

Figure 2. Custom fixture, developed during the project for the Deben in situ tensile/compression stage, that allows compression testing of a small sample while it is submersed in fluid (used for both in- & ex-situ)



Experimentation: With a special fixture (fig. 2), stress-relaxation tests were performed on cylindrical porcine meniscus samples to peak force values of 1.5kN and 4kN and under three conditions:

1. Submersed in PBS[2], simulating the hydrated knee-joint space
2. In Situ, under the ESEM[3] in variable pressure (VP) mode
3. In Situ, also in VP mode and after full drying of the sample

(1) https://orthoinfo.aaos.org/en/diseases--conditions/discoid-meniscus/
(2) phosphate buffered saline, used for its similar properties to the synovial fluid that usually resides in the native meniscus
(3) environmental scanning electron microscope, model: Carl Zeiss Evo LS15

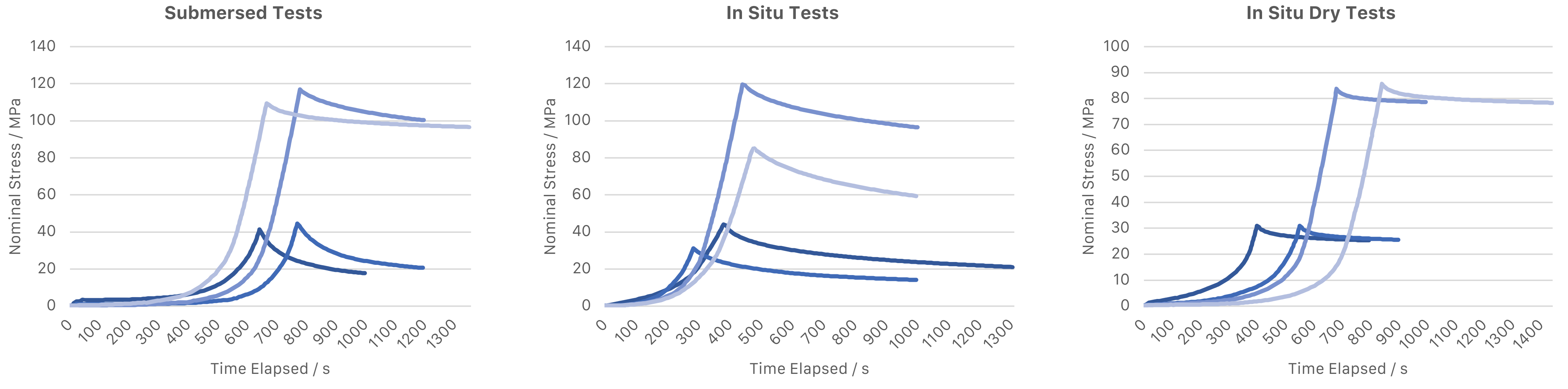
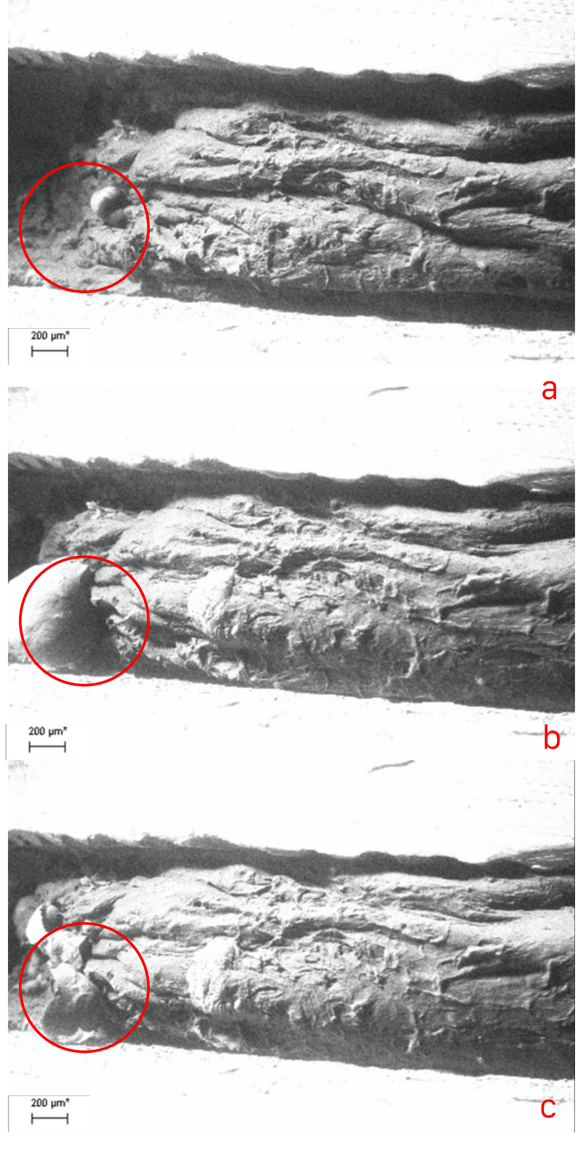


Figure 3. Stress versus time curves for the twelve stress-relaxation tests



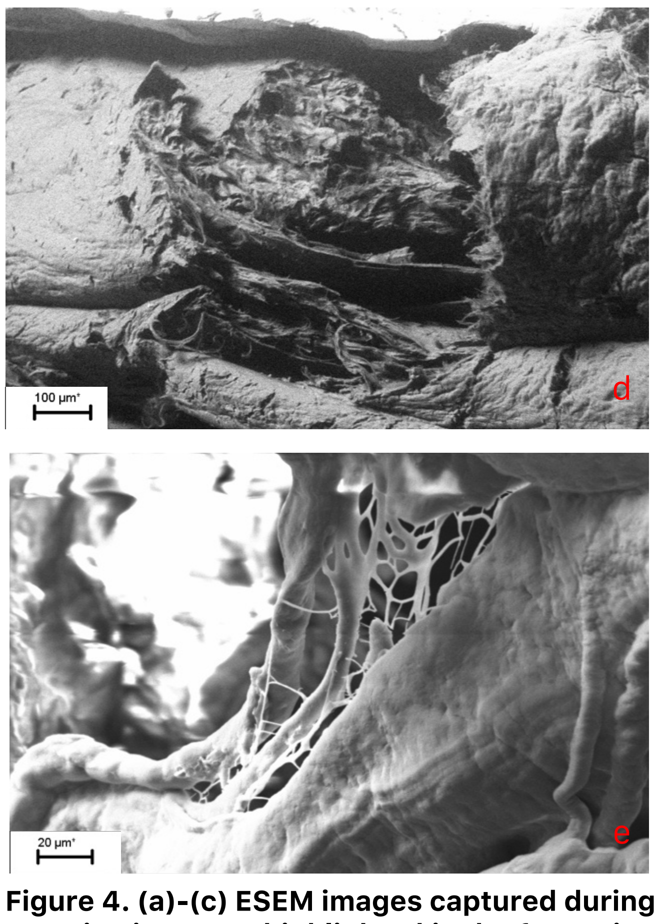


Figure 4. (a)-(c) ESEM images captured during 'wet' in situ test – highlighted is the formation & bursting of a bubble, indicating the presence of significant internal fluid (d) ESEM image captured during dry in situ test showing a radial tear of the collagen matrix (e) SEM high vacuum mode image of Au/Pd sputter coated sample showing collagen fibres separated from their bundles

Results and Conclusions: The stress-relaxation tests were successful in providing insight into the mechanical properties of the meniscus. It is known that meniscal tissue displays viscoelastic, time-dependent behaviour. This project was able to study the change in this behaviour associated with altered tissue hydration. There is a positive correlation between hydration and the rate and amount of relaxation, evident in figure 3. The correlation is more pronounced across the 1.5kN tests, indicating that fluid-based behaviour is more dominant at lower stress. Simultaneous ESEM imaging showed structural deformation and microscopic features, such as tears and bubbles, that would be invisible without magnified inspection. The tears indicate an excess in circumferentially orientated tension. Although higher resolutions are achieved when imaging dry, coated samples in high vacuum (see figure 4(e)), ESEM has been shown to still be an invaluable tool in the testing of biologicals.