There are currently no suitable alternatives for histology in evaluating the quality of cartilage repair tissue (Hoemann et al., 2011). Our group has been focusing on new, non-destructive methods to evaluate cartilage repair through its electromechanical and mechanical properties. While mechanical properties have always been a reliable indicator of the functional properties of cartilage (Armstrong & Mow, 1982), electromechanical properties also provide functional assessment information of cartilage that relates to structure and composition (Sim et al., 2014).

The purpose of this study was to demonstrate the ability of rapid and non-destructive mapping techniques (Arthro-BST and automated indentation) to assess functional properties of repair cartilage and its integration to the surrounding articular surface in a sheep model.

**Methods**

- **Treated samples**
  - Five mature sheep (8-9 year old)
  - Bone marrow stimulation model
  - Sacrificed 9 months post-surgery
- **Control samples**
  - Two mature sheep (8 year old)
  - No surgical intervention

- Articular surfaces were attached to a testing chamber filled with PBS and equipped with a camera-registration system (Fig. 1).
- A position grid was superimposed on the image of the sample (~40 positions/surface).
- Electromechanical properties were obtained ex vivo using the Benchtop version of the Arthro-BST (Fig. 1).
- 1 measurement/position
- The devices calculates a quantitative parameter (QP) through its spherical indenter (radius = 3.2 mm) with an array of 37 microelectrodes (5/mm²). The QP corresponds to the number of microelectrodes in contact with the cartilage when the sum of their streaming potentials reaches 100 mV.
- High QP indicates weak electromechanical properties
- Low QP indicates strong electromechanical properties

**What are Streaming Potentials?**

- During compression, positive mobile ions in the extracellular fluid are discharged relative to the fixed negatively charged collagen matrix.
- Difference in potential is generated across the collagen network (Fig. A) creating normal streaming potentials.
- In degenerated cartilage (Fig. B), the collagen network is disrupted and there is loss of proteoglycans, leading to anomalously low streaming potentials.

**Mechanical properties** were subsequently obtained ex vivo using the automated indentation technique (Fig. 2).

- 1 measurement/position
- The Mach-1 v500csA was used as the mechanical tester with a spherical indenter (r=0.5mm) attached under its multiaxial load cell. This technique detects the perpendicular direction at each position and moves the spherical indenter along this direction (indentation amplitude of 200 μm at 200 μm/s) while measuring the normal component of the resulting force.
- The structural stiffness (load (N) divided by the indentation depth (mm)) was calculated at each position.

- Afterwards, biopsies were taken in the distal repaired defect for compression tests.
- Distal condyles were fixed, decalcified, and paraffin sections stained with Safranin O-Fast Green.
- The histology sections were then associated with the position grid where the electromechanical and mechanical properties were obtained.

**Results**

In control articular surfaces, thicker cartilage showed lower structural stiffness and higher QP values (Fig.3).

- **Region I, II and III**
  - Similar stiffness and QP in control and surgical groups

**Region IV**

- Cartilage tissues outside of the repair site showed slight but significantly lower stiffness and higher electromechanical QP compared to control

- **Region V**
  - Repair cartilage tissues showed significantly higher stiffness and lower electromechanical QP compared to control

**Thickness of the cartilage repair tissue ranged from 0 to 1200 µm (mean thickness 315±200 µm, n=10) compared to ~1100 µm for native cartilage at the edge of the defect.**

- Native cartilage at the edge of the defect showed surface GAG depletion at the defect edges in around half of the operated medial condyles (Fig. 4A, C, black arrows).

- Increasing electromechanical QP and poroelastic behavior along with decreasing structural stiffness parallels increasing quality of the cartilage repair.

**Biopsy compression tests from hyaline to GAG-depleted repair cartilage:**

- Fibril modulus goes from 13.04 Mpa to 0.83 Mpa
- Matrix modulus goes from 0.59 Mpa to 0.02 Mpa
- Permeability goes from 0.0021x10⁻¹² mm²/s to 0.29x10⁻¹² mm²/s

The Jamshidi biopsy used for unconfined compression tests creates missing tissue in some histology sections which complicates histological scoring and histomorphometry of repair tissue in these sections (Fig. 5 vs Fig. 4).

**Conclusions**

- Mechanical characterisation revealed poroelastic cartilage behavior in repair tissues following bone marrow stimulation in aged sheep.
- Slight surface cartilage degradation in the distal condyle which is consistent aged sheep and arthrotomy (Custers et al., 2009; Hoemann et al., 2005).
- Repair tissues had increased mean structural stiffness and lower electromechanical QP that could be partly explained by the thinner repair cartilage.

**References**

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