Non-destructive electromechanical assessment (Arthro-BST) of human articular cartilage correlates with histological scores and biomechanical properties

S. Sim † †, A. Chevrier †, M. Garon †, E. Quenneville †, A. Yaroshinsky §, C.D. Hoemann † †, M.D. Buschmann † † †

Article Info

Article history:
Received 26 March 2014
Accepted 18 August 2014

Keywords:
Articular cartilage
Streaming potentials
Osteoarthritis
Biomechanics
Mankin score
Polarized light microscopy

Summary

Objective: The hand-held Arthro-BST™ device is used to map electromechanical properties of articular cartilage. The purpose of the study was to evaluate correlation of electromechanical properties with histological, biochemical and biomechanical properties of cartilage.

Method: Electromechanical properties (quantitative parameter (QP)) of eight human distal femurs were mapped manually ex vivo using the Arthro-BST (1 measure/site, 5 s/measure, 3209 sites). Osteochondral cores were then harvested from different areas on the femurs and assessed with the Mankin histological score. Prior to histoprocessing, cores were tested in unconfined compression. A subset of the cores was analyzed with polarized light microscopy (PLM) to assess collagen structure. Biochemical assays were done on additional cores to obtain water content and glycosaminoglycan (GAG) content. The QP corresponding to each core was calculated by averaging all QPs collected within 6 mm of the core center.

Results: The electromechanical QP correlated strongly with both the Mankin score and the PLM score ($r = 0.73, P < 0.0001$ and $r = 0.70, P < 0.0001$ respectively) thus accurately reflecting tissue quality and collagen architecture. Electromechanical QP also correlated strongly with biomechanical properties including fibril modulus ($r = -0.76, P < 0.0001$), matrix modulus ($r = -0.69, P < 0.0001$), and log of permeability ($r = 0.72, P < 0.0001$). The QP correlated weakly with GAG per wet weight and with water content ($r = -0.50, P < 0.0003$ and $r = 0.39, P < 0.006$ respectively).

Conclusion: Non-destructive electromechanical QP measurements correlate strongly with histological scores and biomechanical parameters providing a rapid and reliable assessment of articular cartilage quality.

© 2014 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Introduction

The deterioration of articular cartilage is a hallmark of degenerative joint diseases such as osteoarthritis which affects 8.5% of the adult population with 40% prevalence above the age of 70. Currently, joint health and function are diagnosed at a late stage by methods including physical examination, X-ray and magnetic resonance imaging of joint space or visual arthroscopy with a blunt probe. None of these techniques are able to provide diagnostic information early in the disease process.

Multiple research groups have invented devices to assess cartilage function during arthroscopic surgery. Methods involving ultrasound biomicroscopy, arthroscopic ultrasound imaging, optical reflection spectroscopy, pulsed laser irradiation or near-infrared spectroscopy have been proposed. Two devices obtained FDA regulatory clearance (Artscan™ 200 Arthroscopic Cartilage Stiffness Tester and Actaeon™ Probe), while the Artscan...
was only briefly commercialized. The reasons for limited clinical acceptance may be related to challenges in ease of use and difficult sensor orientation relative to cartilage surface as well as the need for repeated indentations at a controlled level of force. There is an enduring demand in Orthopedics for an objective and reliable technique to evaluate articular cartilage tissue health.

Streaming potentials are compression-induced electric potentials that have been shown to reflect the structural and functional integrity of cartilage. Streaming potential are generated by fluid-solid phase interactions in the loaded extracellular matrix, given that proteoglycans are negatively charged and entrapped within the collagen network, while an excess of mobile positive ions exists in the interstitial fluid. Thus, under equilibrium conditions, without no load applied, there is no net macroscopic electric field present since mobile cations are symmetrically arranged around negatively charged proteoglycan. However, when the cartilage is compressed, the flow of the interstitial fluid entrains motion of the positive mobile ions relatively to the fixed negatively charges of the solid phase, generating measurable streaming potentials.

The Arthro-BST™ measures streaming potentials in articular cartilage on 37 microelectrodes located on its spherical indenter during a gentle and instantaneous compression (<1 s). The contact between the indenter and the cartilage is tracked during measurement through the use of a non-planar microelectrode array to measure streaming potentials without the need to control the force used by the surgeon to compress the cartilage. The calculation of the quantitative parameter (QP) is independent of the velocity of indentation or device orientation since the software discards measurements when the loading time is outside the pre-defined limits, corresponding to high and low velocity, in order to minimize the effect of loading velocity on measurements. This device was previously used to assess degenerative changes on equine cartilage subjected ex vivo to high levels of mechanical impact and showed high reliability and excellent agreement between and within users' electromechanical measurements.

Histological scoring, biochemical analyses and biomechanical testing offer precise and specific measurements of cartilage structure and function (more so than MRI and X-ray) but involve destructive processing of tissue, and do not represent the entire joint surface. The objective of this study was to map electromechanical properties of cartilage across entire articular surfaces non-destructively with the hand-held Arthro-BST™ and to relate these maps with histological, biochemical and biomechanical properties of cartilage. Since the structure and composition of articular cartilage are reflected by its electromechanical properties, we hypothesized that the Arthro-BST QP correlates directly with histological, biochemical and biomechanical properties of cartilage. A secondary hypothesis was that the Arthro-BST can precisely assess cartilage quality non-destructively and rapidly. To test these hypotheses, the electromechanical properties of articular surfaces of eight human distal femurs were measured ex vivo with the Arthro-BST and osteochondral cores were then harvested to obtain histological, biochemical and biomechanical properties of cartilage.

**Method**

**Sample source and preparation**

Frozen cadaveric human distal femurs from research donors (n = 8; <80°C; age range 35–43 years old; three females and five males; four left joints and four right joints) were provided by a tissue bank (RTI Surgical, Florida, USA). The articular surfaces were thawed in a plastic bag overnight at 4°C. The distal femur was cut through a horizontal plane with a band saw at the appropriate orientation to permit mounting in a chamber for electromechanical mapping. Distal femurs were fixed onto a cylindrical platform (D = 85 mm) and the platform with the attached femur was then fixed to a testing chamber (D = 190 mm, H = 100 mm) equipped with a camera (1280 x 960 pixels) and a positioning software (Mapping Toolbox software, Biomomentum Inc.) The testing chamber was filled with phosphate buffered saline (pH 7.4) and a minimum of 15 min was allowed for equilibration prior to electromechanical mapping of the trochlea and anterior/central condyles (details below). Following core extraction, the central/posterior condyles were removed with a second band saw cut and the platform to the testing chamber for mapping of the central/posterior condyles followed by core extraction (details below). None of the donors had documented joint pathologies, however shallow focal cartilage lesions were observed on the articular surfaces of four out of eight femurs [see trochlear lesion in Fig. 1(E)].

**Arthro-BST mapping**

The Arthro-BST™ (Biomomentum Inc.) measures streaming potentials generated during a rapid compression of the articular cartilage with an array of microelectrodes lying on a semi-spherical indenter (effective radius of the tip = 3.18 mm, 5 microelectrodes/mm²). The device calculates a quantitative parameter (QP, arbitrary units) of cartilage electromechanical activity corresponding to the number of microelectrodes in contact with the cartilage when the sum of their streaming potential reaches 100 mV. A high QP therefore indicates weak electromechanical properties and poor load-bearing capacity and low QP indicates strong electromechanical properties and high load-bearing capacity. Using the bench top version of the Arthro-BST, a positioning software overlays a 25 columns x 19 rows position grid (corresponding to ~9 sites per cm² on the articular surface) on the live video feed to help measurement registration and create a uniform mapping. The spherical indenter of the Arthro-BST was manually compressed onto the cartilage surface for about 1 s at each position of the grid and the device displayed and recorded the corresponding QP.

**Core extraction**

Following a macroscopic visual assessment of the articular surfaces, a total of 163 osteochondral cores were harvested from non-lesional and also from lesional regions in triplicate (histology, biomechanics and biochemistry). Lesional areas appeared only sporadically in the age range examined here (35–43 years) so most of the cores were from non-lesional regions. Osteochondral cores (length > 10 mm) were harvested using Smith and Nephew tubular chisels of 4.5 mm diameter (for histology) and 3.5 mm diameter (for biomechanical and biochemical analyses). Cores for histology were fixed in 10% neutral buffered formalin. After coring, the condyles and trochlea were placed back onto the testing chamber, visually repositioned and oriented as per the initial position and a second image was acquired to precisely (~1 mm) document the location of each core relative to the position grid used for Arthro-BST measurements. The Arthro-BST’s electromechanical QP corresponding to the cored site was calculated as the average of all QPs measured within 6 mm from the core center location and was between 1 to a maximum of 4 QP measures. In total, 59 cores were isolated for histological assessment only, 53 cores for biochemical analysis and 51 cores for biomechanical testing followed by histology.
Histoprocessing and staining

Each sample for histology was decalcified in 0.5 N HCl/0.1% glutaraldehyde. Samples were dehydrated and cleared in ethanol and xylene, infiltrated with paraffin and embedded in paraffin. Then, 5 μm paraffin sections were obtained with a RM2155 (Leica) motorized microtome and collected on Superfrost plus slides. The sections were stained with Safranin O-Fast Green and scored with...
the Mankin histological-histochemical grading system28 by one blinded observer. Furthermore, mounted unstained sections from 68 of the cores were scored by two blinded observers using a validated polarized light microscopy (PLM) qualitative score for collagen structure29, where a score of 5 indicates that collagen is stratified into three distinct zones (superficial, transitional and radial) of the correct proportions and birefringence properties while a score of 0 indicates that the collagen is completely disorganized.

**Biochemical testing**

Water content, glycosaminoglycan (GAG), collagen and DNA content per wet weight and dry weight were determined as previously published30,31. Briefly, full thickness cartilage biopsies \( (n = 53) \) were thawed on ice, weighed, lyophilized for 24 h and re-weighed to obtain water content. Samples were incubated in 125 \( \mu \)L papain digestion cocktail in sterile l-cysteine/phosphate buffer EDTA (PBE) \( (50 \, \mu \)L digestion cocktail per mg of biopsy wet weight) for 16 h at 60 °C. PicoGreen assay was performed on duplicate samples of the papain digest and DNA content obtained with a standard curve of calf thymus DNA with chondrocyte DNA content set at 3.7 pg per cell.28 The DMMB assay32 was performed on duplicate samples of the papain digest and GAG content was obtained with a standard curve of shark Chondroitin Sulfate C sodium salt. Papain digested samples were also hydrolyzed with HCl at 110 °C for 18 h and the hydroxyproline (HPR)33 assay was then performed on duplicate samples to obtain collagen content using a standard curve of trans-4-hydroxy-L-proline and a conversion factor of 13.15%.

**Biomechanical testing**

The thickness of the cartilage layer of each osteochondral core \( (D = 3.5 \, \text{mm}) \) was measured under a calibrated dissection microscope [Fig. 2(C)] as the average of six measurements at two different angles using the Northern Eclipse software version 8 (EMPIX Imaging Inc., Mississauga, Canada). Each core was thawed and equilibrated in PBS for at least 20 min prior to thickness measurement and was then gripped by the bone portion into a threaded core sample holder leaving the cartilage layer completely outside of the grip [Fig. 2(D)]. This sample holder assembly was fixed to the bottom of a testing chamber \( (D = 100 \, \text{mm}, H = 50 \, \text{mm}) \), filled with PBS and mounted onto a Mach-1 mechanical tester (Biomomentum Inc.). Cores were tested in unconfined compression using stress relaxation (precompression of 10% of thickness followed by ve compressions each of 2% of thickness). Cores were tested in unconfined compression using stress relaxation (precompression of 10% of thickness followed by ve compressions each of 2% of thickness). The 2% stress relaxation ramps were individually buffered EDTA (PBE) \( (50 \, \mu \)L digestion cocktail per mg of biopsy wet weight) for 16 h at 60 °C. PicoGreen assay was performed on duplicate samples of the papain digest and DNA content obtained with a standard curve of calf thymus DNA with chondrocyte DNA content set at 3.7 pg per cell.28 The DMMB assay32 was performed on duplicate samples of the papain digest and GAG content was obtained with a standard curve of shark Chondroitin Sulfate C sodium salt. Papain digested samples were also hydrolyzed with HCl at 110 °C for 18 h and the hydroxyproline (HPR)33 assay was then performed on duplicate samples to obtain collagen content using a standard curve of trans-4-hydroxy-L-proline and a conversion factor of 13.15%.

**Statistical analysis**

The relationships between the QP and histological, biochemical or biomechanical parameters were assessed by parametric correlation analyses using Pearson’s correlation coefficient \( (r) \); 95% two-sided confidence intervals for correlation coefficients were calculated. A multiple regression analysis was performed using a mixed effects model, with QP as the response variable, to examine the relationships between the biomechanical QP and each family of independent variables — histological, biochemical and biomechanical. Note that these best fit equations have not been cross-validated using another data set. For biochemical parameters, independent Student’s t-tests were performed to examine the difference between the means of lesional and non-lesional regions. For all statistical tests, a \( P \)-value of 0.05 (two sided) or smaller was considered statistically significant. Statistical analyses were performed with SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

**Results**

**Electromechanical QP correlates with histological scores**

The total Mankin score (scale 0 for normal cartilage to 14 for totally degraded cartilage) was on average 1 for non-lesional cores (82% of cores) and 5 for lesional cores, indicating that only mild to moderate cartilage degeneration was present occasionally on the femurs. For analyses, the cores were stratified into three Mankin score groups \( (0–2, 3–5 \text{ and } 6–9) \). For the first group (Mankin 0–2), the histological slides were normal or had a slight roughening of the cartilage surface [Fig. 3(A)]. For the intermediate group (Mankin 3–5), decreased Safranin O staining and structural alterations were apparent in the superficial zone [Fig. 3(B)]. Clefts and GAG depletion as assessed by reduced Safranin O staining were observed for the most degenerated group (Mankin 6–9) [Fig. 3(C)]. Similarly, cores assessed by the PLM collagen score (scale 0 (entirely disorganized) to 5 (normal)) were divided into two groups: PLM score 3–5 [Fig. 3(D)] which all have three distinct zones of collagen structure (as in superficial, transitional, radial) and a PLM score of 0–2 [Fig. 3(E)] where less than three zones were apparent. Table I shows that a positive correlation was found between electromechanical QP and Mankin score \( (r = 0.73, P < 0.0001, \text{Table I}) \), while a negative correlation was observed between QP and PLM score \( (r = -0.70, P < 0.0001, \text{Table I}) \). A multiple regression analysis revealed that the electromechanical QP is more closely related to the Mankin histological score \( (P < 0.0001, \text{Table II}) \) than to the PLM score \( (P = 0.6232, \text{Table II}) \).

**Electromechanical QP weakly correlates with GAG and water content**

As expected, there was a significant increase in water content in cores extracted from lesional regions compared to non-lesional regions (average water content was 82 ± 4% \( (N = 6) \) vs 74 ± 4% \( (N = 42) \) respectively; \( P = 0.0002 \) with an independent Student’s t-test). The cores collected from lesional regions also had decreased GAG per wet weight content (average was 24 ± 13 \( \mu \)g/mg \( (N = 6) \) in lesional vs 44 ± 13 \( \mu \)g/mg \( (N = 42) \) in normal areas; \( P = 0.0009 \) with an independent Student’s t-test) and GAG per dry weight content (average was 116 ± 37 \( \mu \)g/mg \( (N = 6) \) in lesional vs 163 ± 52 \( \mu \)g/mg \( (N = 41) \) in normal areas; \( P = 0.04 \) with an independent Student’s t-test). Collagen content and cell density were similar in lesional and non-lesional regions (average collagen content was 0.11 ± 0.03 mg/mg \( (N = 43) \) wet weight or 0.45 ± 0.12 mg/mg \( (N = 43) \) dry weight with 16,400 ± 3400 cells/mg \( (N = 48) \) wet weight or 68,300 ± 22,000 cells/mg \( (N = 48) \) dry weight). There was a weak correlation of the electromechanical QP with increasing water content \( (r = 0.39, P = 0.006; \text{Fig. 4(A), Table I}) \). Additionally, there was a weak correlation of the electromechanical QP with increasing GAG content (chondroitin sulfate) per wet weight \( (r = -0.50, P = 0.0003; \text{Fig. 4(B), Table I}) \) and between the electromechanical QP and GAG content per dry weight \( (r = -0.31, P = 0.0316, \text{Table I}) \). There was no correlation between electromechanical QP and the other biochemical parameters: collagen content per wet and dry
weight and number of cells per wet and dry weight [Fig. 4(C and D), Table I]. The multiple regression analysis showed that the water content \( (P = 0.0070, \text{Table II}) \) was more significant in modeling QP than the GAG content per dry weight \( (P = 0.1305, \text{Table II}) \).

Electromechanical QP correlates with biomechanical properties

A high QP indicates cartilage degeneration with weaker electromechanical properties which relate to biomechanical weakening revealed by lower modulus and higher hydraulic permeability. Following the normality test, the natural logarithm of permeability had a more normal distribution than permeability itself. Also, its regression with QP was more linear than permeability. Thus the natural logarithm of permeability was used for subsequent statistical analysis (Table I). Higher QP values were correlated strongly with decreasing \( E_f \) \( [r = -0.76, P < 0.0001; \text{Fig. 5(A), Table I}] \) and \( E_m \) \( [r = -0.69, P < 0.0001; \text{Fig. 5(B), Table I}] \), and increasing permeability \( (\log k)) [r = 0.72, P < 0.0001; \text{Fig. 5(C), Table I}] \). The cartilage samples had an average thickness of \( 2.67 \pm 0.47 \text{ mm} \) (obtained with a calibrated dissection microscope) and no correlation was found between the electromechanical QP and the thickness \( [r = 0.08, P = 0.5852; \text{Fig. 5(D)}] \). The multiple regression analysis showed that the electromechanical QP was most closely related to \( E_f \) \( (P = 0.0012, \text{Table II}) \) and the natural logarithm of permeability \( (P = 0.0394, \text{Table II}) \) than \( E_m \) \( (P = 0.4510, \text{Table II}) \).

### Table I

<table>
<thead>
<tr>
<th>Parameters</th>
<th>QP</th>
<th>Pearson's correlation ( r )</th>
<th>95% C.I. lower</th>
<th>95% C.I. upper</th>
<th>( P )-value &lt;= 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomechanical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibril modulus ( E_f )</td>
<td>-0.76</td>
<td>-0.8563</td>
<td>-0.6053</td>
<td></td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Matrix modulus ( E_m )</td>
<td>-0.69</td>
<td>-0.8117</td>
<td>-0.5035</td>
<td></td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Permeability ( k )</td>
<td>0.46</td>
<td>0.2025</td>
<td>0.6346</td>
<td></td>
<td>( P = 0.0008 )</td>
</tr>
<tr>
<td>Natural logarithm of permeability ( \log k )</td>
<td>0.72</td>
<td>0.5380</td>
<td>0.8272</td>
<td></td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.08</td>
<td>-0.2024</td>
<td>0.3457</td>
<td></td>
<td>( P = 0.5852 )</td>
</tr>
<tr>
<td>Histological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mankin score</td>
<td>0.73</td>
<td>0.6333</td>
<td>0.8090</td>
<td></td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>PLM score</td>
<td>-0.70</td>
<td>-0.8015</td>
<td>-0.5487</td>
<td></td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Biochemical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water content</td>
<td>0.39</td>
<td>0.1169</td>
<td>0.6055</td>
<td></td>
<td>( P = 0.006 )</td>
</tr>
<tr>
<td>GAG per wet weight</td>
<td>-0.50</td>
<td>-0.2471</td>
<td>-0.6841</td>
<td></td>
<td>( P = 0.0003 )</td>
</tr>
<tr>
<td>GAG per dry weight</td>
<td>-0.31</td>
<td>-0.5490</td>
<td>-0.0260</td>
<td></td>
<td>( P = 0.0316 )</td>
</tr>
<tr>
<td>Collagen per dry weight</td>
<td>0.18</td>
<td>-0.1316</td>
<td>0.4521</td>
<td></td>
<td>( P = 0.2543 )</td>
</tr>
<tr>
<td>Number of cells per wet weight</td>
<td>0.15</td>
<td>-0.1415</td>
<td>0.4152</td>
<td></td>
<td>( P = 0.3083 )</td>
</tr>
</tbody>
</table>

Fig. 3. (A, B, C) Representative Safranin O/Fast Green stained sections for three groups of the Mankin score and the corresponding electromechanical QP (mean ± SD, \( N = 110 \) cores); (D, E) Representative PLM slides for two groupings of the PLM score and the corresponding QP (mean ± SD, \( N = 68 \) cores). Bars = 1 mm.
Discussion

The purpose of this study was to relate measurements obtained with a hand-held electromechanical device (Arthro-BST™) that maps electromechanical properties of cartilage across an entire surface non-destructively, to histological, biochemical and biomechanical properties of osteochondral cores harvested at different locations from eight human distal femurs. The electromechanical QP correlated strongly with the Mankin score (Fig. 3), the PLM score of collagen organization (Fig. 3) and with uncon fined compression mechanical parameters (Fig. 5); while weaker correlations were observed with the biochemical composition (GAG per dry or wet weight) and water content (Fig. 4). The secondary hypothesis that the Arthro-BST provides precise and rapid non-destructive assessments of cartilage quality was also borne out by these correlations and the fact that the electromechanical mapping of an entire articular surface takes around 30 min to complete while histological, biochemical or biomechanical characterizations require several days or weeks and only provide information on specific locations that are consumed by the analyses.

This study confirmed the hypothesis that electromechanical QP is strongly related to the structure and organization of the collagen network and to GAG content revealed by PLM and Safranin–O–Fast Green staining, respectively. This relationship is consistent with prior work where a disorganised structure in degenerated cartilage was related to streaming potentials. It is important to note that previous work reported the streaming potentials measurements as streaming potential integral (SPI) while in the present study; it is reported as a QP which reflects the number of microelectrodes in contact with the articular cartilage when the sum reaches 100 mV. The new parameter QP is consequently inversely proportional to SPI. The new parameter QP has significant advantages vs SPI including the simplicity of calculation and robustness to noise. As reported by others, the proteoglycan content decreases rapidly relative to the collagen content during the progression of OA, but while the collagen content is maintained, its organization and integrity is severely perturbed. Those changes of collagen organization in articular cartilage are revealed by PLM where a strong correlation between the PLM score and the electromechanical assessment was found here. In the multiple regression analysis, the Mankin score was more closely related to QP than PLM, possibly since the Mankin score accounts for more than just collagen related features when assessing matrix integrity. Nonetheless both histological assessments were strongly related to electromechanics where a lower electromechanical QP indicates better quality articular cartilage; corresponding to a lower Mankin score (Fig. 6) and a higher PLM score.

Biochemical composition is also expected to be reflected in the electromechanical QP. In our study QP correlated positively with water content since the disruption of the collagen network leads to GAG loss and tissue swelling and increased water content.

Table II

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Model QP</th>
<th>Coefficient estimates of fixed effects</th>
<th>95% C.I. lower</th>
<th>95% C.I. upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomechanical</td>
<td>QP = a<em>E_f + b</em>Em + c*logk</td>
<td>a = -0.1527</td>
<td>-0.241</td>
<td>-0.064</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b = -0.0838</td>
<td>-2.520</td>
<td>1.142</td>
<td>0.4510</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c = 1.0720</td>
<td>0.055</td>
<td>2.089</td>
<td>0.0394</td>
</tr>
<tr>
<td>Histological</td>
<td>QP = d<em>Mankin score + e</em>PLM score</td>
<td>d = 1.0699</td>
<td>0.6600</td>
<td>1.4799</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e = -0.2415</td>
<td>-1.2200</td>
<td>0.7371</td>
<td>0.6232</td>
</tr>
<tr>
<td>Biochemical</td>
<td>QP = f<em>water content + g</em>GAG per dry weight</td>
<td>f = 0.2075</td>
<td>0.0604</td>
<td>0.3546</td>
<td>0.0070</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g = -0.0115</td>
<td>-0.0266</td>
<td>0.0036</td>
<td>0.1305</td>
</tr>
</tbody>
</table>

Fig. 4. (A) Positive correlation between electromechanical QP and water content; (B) Negative correlation between electromechanical QP and GAG content (chondroitin sulfate per wet weight); (C) No correlation between electromechanical QP and collagen per dry weight; (D) No correlation between electromechanical QP and number of cells per wet weight.
from degeneration. Moreover, proteoglycan loss is one of the early signs of cartilage degradation and QP also correlated with GAG content that are the source of negative fixed charge. Simple collagen content was not related to the electromechanical QP as it is more the integrity of the collagen network rather than its content that changes with degeneration. Effectively, in the early degeneration phase of articular cartilage, the collagen content is initially maintained but there is severe disruption in the collagen architecture. Moreover, our measured biochemical composition for non-lesional articular cartilage fall in close agreement with previously published values. Our findings show that electromechanical measurements are sensitive to abnormally high tissue water content and corresponding lower fixed charge density, which are important characteristics of early cartilage degradation.

In addition to histological features and biochemical composition, electromechanical QP correlated with unconstrained compression mechanical properties, as expected, since streaming potentials are known to reflect the structure and composition of articular cartilage. Osteochondral cores with a high fibril modulus have excellent integrity of the extracellular matrix (confirmed by histology) and strong electromechanical properties (with QP between 6 and 7). In the multiple regression analysis, the effect of $E_f$ on the electromechanical QP was predominant compared to the effect of $E_m$ or $\log k$. Since the electromechanical QP measurement consists of a near instantaneous indentation, this result is consistent with previously published studies where the response to instantaneous compression was mainly controlled by the integrity of the collagen network.

The correspondence of the electromechanical QP to histological, biomechanical and biochemical parameters can be summarised visually on a logarithmic scale. It should be mentioned that the scale of the electromechanical QP could reach 30 suggesting a wider dynamic range than the other parameters. This would allow highlighting even higher levels of degradation that are not seen in this study that uses mostly healthy non-lesional cartilage. This direct relationship between the electromechanical QP and histological scores, fibril modulus $E_f$ and GAG content support the use of this non-destructive electromechanical assessment as a surrogate for destructive and time-consuming assessments of articular cartilage properties.

In the interpretation of our data, it is also important to keep in mind that about 90% of the area of the articular surfaces (82% of the cores) was non-lesional. It is expected that the inclusion of more degenerated articular surfaces in the study would have strengthened the observed correlations. Also, the inclusion of physiological parameters such as age, gender, BMI or smoking habits could further specify a more general model relating electromechanical QP to cartilage and patient-specific parameters.

One limitation of the Arthro-BST is that for low QP ($<4$), it is difficult to distinguish exceptionally high electromechanical properties of the cartilage from an extreme thinness due to the geometry of the indenter and its limitations for thin cartilage. One solution to address this issue is that a very low QP will be compared to a reference QP map, and be categorized as degenerated if it is much lower than normal, thus indicating thinness rather than high electromechanical properties. This particular limitation of the device caused no problem in the current study since the lowest average QP obtained was 6. Nonetheless, it will be important to account for in studies of cartilage repair where the repaired cartilage may be thinner than normal cartilage. We also found that the electromechanical QP did not correlate with thickness, which is reasonable since the indenter force was previously found to be independent of thickness for thickness ranging from 2 to 4 mm, in contrast to measurements on thinner...
cartilage where a correlation between the streaming potentials and the thickness was seen. These results suggest that the non-destructive evaluation of cartilage electromechanical properties by the Arthro-BST is more sensitive to the integrity and the structure of the extracellular matrix. Considering the fact that the non-destructive mapping of an entire distal femur with a high resolution (about 300 measures per distal femur) takes about 30 min, the Arthro-BST can provide a rapid and reliable tool for cartilage assessment where spatially resolved measurements over the entire surface are desired. For example, the Arthro-BST could be useful in the quantitative evaluation and mapping of the electromechanical properties of entire articular surfaces in cartilage repair studies or studies of wear-patterns in osteoarthritis. Moreover, the device could be useful in cartilage research in general to further understanding of cartilage diseases, and to develop new therapeutic products, cartilage repair techniques and reliable animal models of osteoarthritis. Additionally, since that the Arthro-BST has been designed for compatibility with arthroscopy, it could reveal itself useful in assessing the cartilage quality during surgery and could aid in the establishment of treatment algorithms.

Author contributions

Sotcheadt Sim: study design, electromechanical measurements, biomechanical measurements, analysis and interpretation of data, statistical analysis, literature review, drafting and revision of the article.

Anik Chevrier: study design, histoprocessing, sectioning and staining, histological scoring, biochemical assays and revision of the article.

Martin Garon: study design, preliminary histological statistical analysis and revision of the article.

Eric Quenneville: study design, preliminary histological statistical analysis and revision of the article.

Alex Yaroshinsky: Statistical expertise and revision of the article.

Caroline D. Hoemann: Histological and biochemical study design and revision of the article.

Michael D. Buschmann: study design, analysis and interpretation of data, statistical expertise and revision of the article.

All authors have read and provided final approval of the manuscript.

Role of the funding source

Operating grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and from Biomomentum Inc. paid for all experimental procedures, graduate student stipends, salary support for research staff, and towards presentation of findings at the International Cartilage Repair Society World Congress (September 2013, Izmir, Turkey) and the Orthropeadic Research Society 2014 Annual Meeting (March 2014, New Orleans, LA, USA). The National Sciences and Engineering Research Council (NSERC), the Fonds de recherche du Québec – Nature et technologies (FRQNT) and Biomomentum Inc. funded Sim Sotcheadt scholarship. The FRQ-Santé Groupe de Recherche en Sciences et Technologies Biomédicales (GRSTB) funds provided histology service.

Conflict of interest

E Quenneville and M Garon are the owners of Biomomentum Inc.

Acknowledgments

We acknowledge the technical contributions of Geneviève Picard, Gabrielle Picard, Hubert Camirand, Marie-Hélène Boulanger and Sylvain Gaufrès. Funding provided by the National Sciences and Engineering Research Council (NSERC) (CRD PJ 445265 12), the Fonds de recherche du Québec – Nature et technologies (FRQNT) and Biomomentum Inc.

References


38. Kleeman RJ, Krockor D, Cedraro A, Tuischer J, Duda GN. Altered cartilage mechanics and histology in knee


