Electromechanical Probe and Automated Indentation Maps Are Sensitive Techniques in Assessing Early Degenerated Human Articular Cartilage

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Abstract: Recent advances in the development of new drugs to halt or even reverse the progression of Osteoarthritis at an early-stage requires new tools to detect early degeneration of articular cartilage. We investigated the ability of an electromechanical probe and an automated indentation technique to characterize entire human articular surfaces for rapid non-destructive discrimination between early degenerated and healthy articular cartilage. Human cadaveric asymptomatic articular surfaces (four pairs of distal femurs and four pairs of tibial plates) were used. They were assessed ex vivo: macroscopically, electromechanically, (maps of the electromechanical quantitative parameter, QP, reflecting streaming potentials), mechanically (maps of the instantaneous modulus, IM), and through cartilage thickness. Osteochondral cores were also harvested from healthy and degenerated regions for histological assessment, biochemical analyses, and unconfined compression tests. The macroscopic visual assessment delimited three distinct regions on each articular surface: Region I was macroscopically degenerated, region II was macroscopically normal but adjacent to regions I and III was the remaining normal articular surface. Thus, each extracted core was assigned to one of the three regions. A mixed effect model revealed that only the QP (\(p < 0.0001\)) and IM (\(p < 0.0001\)) were able to statistically discriminate the three regions. Effect size was higher for QP and IM than other assessments, indicating greater sensitivity to distinguish early degeneration of cartilage. When considering the mapping feature of the QP and IM techniques, it also revealed bilateral symmetry in a moderately similar distribution pattern between bilateral joints.

Keywords: articular cartilage; mechanics; streaming potentials; osteoarthritis; cartilage diagnostic

Early detection of cartilage degeneration is an imperative, since Osteoarthritis evolves slowly over decades permitting a wide timeframe to halt or reverse the disease. The primary changes in the degenerative process of articular cartilage consist of alterations in the structure and biochemical content of cartilage extracellular matrix. There is an urgent need for a quantitative, sensitive, user-independent, and non-destructive tool to detect early cartilage changes to understand the disease process, identify adequate disease-modifying treatments, and evaluate their efficacy.

Benchtop techniques currently used for ex vivo cartilage assessment include visual macroscopic evaluation, histological scores, biochemical assays, and simple mechanical tests. Unfortunately, most of these techniques require isolation of cartilage cores, which leads to partial destruction of the articular surface and possible creation of mechanical and histological artefacts, in addition to being time-consuming. Moreover, information obtained at a single location may not reflect the properties of the entire articular surface, given the spatial variation of cartilage properties. Visual macroscopic evaluation has been subjected to many critiques involving poor inter-observer agreement and incomplete validation to diagnose cartilage lesions. Mechanical and electromechanical properties have been recognized as accurate indicators of the functional properties of cartilage, provided by structure and composition. However, reliable instruments for practical evaluation of these properties over entire surfaces are currently lacking.

Our research group has developed two unique approaches in the quantitative assessment of cartilage that overcome some limitations of other techniques. The first technique uses an arthroscopic probe to measure electromechanical properties of cartilage via compression-induced streaming potentials. The second uses a multiaxial mechanical tester to perform automated spherical indentation and thickness measurements to map mechanical properties. Both approaches offer several advantages compared to other techniques such as being non-destructive, allowing repeated measurements, and performing multiple characterizations at the same location in a short time. Furthermore, the electromechanical probe reliability to evaluate allograft quality has been published recently.

The aim of the current study was to investigate the sensitivity of these two techniques to distinguish...
between cartilage regions that appears macroscopically normal, but are adjacent to visually degenerated regions (where early or mild degeneration is suspected) and cartilage far away from visually degenerated regions (where no degeneration is suspected). These performance results were then compared to those of current gold standard techniques namely macroscopic assessments (adapted from the ICRS grading), cartilage thickness, histological Mankin scores, unconfined mechanical compression, and biochemical assessments. Based on the assumption that patterns in the distribution of mechanical, electromechanical properties, and thickness should be more similar within-donor, this study also aimed to quantitatively evaluate the performance of these non-destructive characterization mapping techniques.

METHODS

Tissue Source

Four pairs of distal femurs (two females and two males, aged 46–64 years) and four pairs of tibial plateaus (one female and three males, aged 52–64 years) from cadaveric human research donors were provided by a tissue bank (ITRI Surgical, Alachua, FL) and stored at −80°C. All samples were handled according to approved institutional ethics committee-certificates.

Study Design

Figure 1 summarizes the study design. Frozen articular surfaces were thawed in a plastic bag at 4°C overnight. A camera-registration system (Biomomentum Inc., Laval, Canada) was first used to superimpose a position grid (~9 positions/cm²; ~120 positions for each distal femur and ~140 positions for each tibial plateau) on the real-time video stream of the entire articular surface. Then, a mapping using the Arthro-BST™ (Biomomentum) was performed to obtain electromechanical properties (30 s/position) followed by a macroscopic assessment (adapted from the ICRS grading) on each sample. Afterward, the automated indentation mapping was performed in order to extract mechanical properties of the articular cartilage at each position of the grid (1 min/position). Osteochondral cores were then extracted randomly from visually abnormal and normal regions for biochemical assays and unconfined compression (UC) tests followed by histological assessment. Cores for biochemistry were flash-frozen; those destined for unconfined compression were stored at −80°C and those for histology were fixed in 10% neutral buffered formalin. Finally, automated thickness mapping was performed at each position of the grid (15 s/position). The thickness mapping was performed after coring to avoid puncture holes in the osteochondral cores used for subsequent tests.

Electromechanical Probe Mapping

Electromechanical properties were mapped ex vivo using the benchtop version of the hand-held medical device Arthro-BST (Biomomentum) as described previously. The device calculates a quantitative parameter (QP), which corresponds to the number of microelectrodes in contact with the cartilage when the sum of their streaming potential reaches 100 mV (inversely proportional to electromechanical activity). The semi-spherical indenter of the device was manually compressed onto the cartilage surface for approximately 1 s, once, at each position of the grid and the device recorded the corresponding QP.

Visual Assessment

Entire articular surfaces were visually assessed with a methodology adapted from the ICRS lesion classification system, where visually normal cartilage corresponds to an ICRS grade of 0. All other grades were considered visually abnormal. The extent of each visually normal and abnormal regions were encircled on pictures of the articular surface with positioning grid superimposed. This visual assessment allowed defining three distinct regions on the articular surface (Fig. 1). First, the encircled region(s) of all abnormal cartilage was/were considered as region I. Then, region II was defined by delimiting the area surrounding region I. Finally, region III was defined as the remaining normal articular surface. All regions occurred at different locations and in different size areas on each distal femur while for tibial plateaus, those regions were mostly at the same location but varied in size.

Automated Indentation Mapping

Mechanical properties were mapped ex vivo, using a technique allowing for automated normal alignment and indentation mapping of materials using a multiaxial mechanical tester Mach-1 v500css (Biomomentum). The technique precisely detects the surface orientation at each position and records the normal load with a multiple-axis load cell (70 N range and 3.5 mN resolution on the vertical axis, 50 N range and 2.5 mN resolution on the horizontals axes). Perpendicular indentation was obtained by simultaneously moving the three stages of the tester at different speeds in order to move the spherical indenter (6.35 mm diameter) perpendicularly to the surface. The cartilage was indented by 200 μm at 500 μm/s for distal femurs and at 200 μm/s for tibial plateaus.

Osteochondral Core Harvesting

Osteochondral cores were harvested using tubular chisels with a 3.5 mm diameter (Smith & Nephew, Memphis, TN). After the extraction of cores, the articular surface was repositioned into the testing chamber to photodocument the location of each core site relative to the position grid used for mapping.

Automated Thickness Mapping

Thickness was mapped with the needle technique by replacing the spherical indenter with a 26G 3/8” Precision-Glide intradermal bevel needle (BD, Franklin Lakes, NJ). The mechanical tester vertically moves the needle toward the sample at a constant speed until it penetrates the cartilage surface and stops inside the bone.

Osteochondral Cores Analyses

First, a calibrated dissecting microscope with Northern Eclipse software version 8 (Empix Imaging Inc., Mississauga, Canada) was used to measure the thickness of the cartilage layer of each osteochondral core. UC consisted of a series of stress relaxations (one precompression of 10% strain and five compressions of 2% strain each with ramp velocity of 0.4% strain/s; relaxation stops when relaxation rate of 0.05 N/min is reached). The 2% stress relaxation ramps were fit (with disk radius = 1.75 mm, deformation = 0.02, effective Poisson’s ratio = 0) to the linear fibril-network-reinforced biphasic model using the Mach-1 Analysis software (Biomomentum). Intrinsic parameters from each fit of the model were the fibril modulus \( E_f \) (MPa), the matrix modulus \( E_m \) (MPa) and the hydraulic permeability \( k \) (10⁻¹² m²/N·s). All osteochondral cores that were tested in UC were then assessed histologically.
For this, each osteochondral core was fixed in 10% NBF and decalcified in 0.5 N HCl/0.1% glutaraldehyde prior to dehydration, clearing and paraffin embedding. 5 μm paraffin sections were obtained and collected on Superfrost plus slides. The sections were stained with Safranin-O/Fast Green and scored with the Mankin histological-histochemical grading system twice by one blinded observer. The average of both measurements was used for analysis. In total, 66 cores were used for histological assessment only and 67 cores for mechanical testing followed by histology. One hundred cores were tested through biochemical assays to obtain water content, glycosaminoglycan (GAG), collagen, and DNA content per wet weight and dry weight using previously published methods.

**Data Processing**

From the automated thickness mapping results, cartilage thickness was calculated at each position by the difference between the vertical position of the surface (where the load starts to increase) and that of the cartilage/bone interface (corresponding to the first inflection point in the displacement/force curve) (Fig. 1, thickness panel). The instantaneous modulus (IM) at each position was obtained by fitting the load-displacement curve (with corresponding thickness and effective Poisson ratio of 0.5) to an elastic model in spherical indentation.

For the compliance of the testing system, the normal indentation amplitude is less than actually, thus data must be processed to account for this. The compliance of the measurement system was characterized using an experimental setup which consists of a stainless steel cylinder (radius of 25.4 mm). Perpendicular indentations were done on the cylinder in order to obtain an experimental curve for the compliance of the system. In order to compare QP and IM maps with biochemical, histological, and UC parameters on cores, the QP and IM corresponding to each core was calculated as the average of all values (using 1–4 measurements) measured within 6 mm from the core center (Fig. 1).

**Statistical Analyses**

All statistical analyses were performed with SAS version 9.3 (SAS Institute Inc., Cary, NC). The intraclass correlation coefficient was obtained for the histological Mankin score as ICC(2,1). Relationships between QP, IM, biochemical, histological, and UC results were assessed by parametric correlation analyses using Pearson’s correlation coefficient (r). In order to assess the performance of each characterization type using uniform statistical sampling, only core assessments were used (Fig. 1). Multiple regression was performed using a mixed effects model to examine which characterization...
could reveal a difference between regions I, II, and III. The core characterization of interest (QP, IM, thickness, Mankin score, UC, or biochemical parameters) was the response variable, the three regions were considered as the explanatory variables (fixed effects) and the donor as the random effect. Effect size (difference between each region means divided by the pooled standard deviation) was calculated for each characterization to reveal their sensitivity to differentiate the three regions and does not take the within donor correlations into account. For all statistical tests, a p-value of 0.05 (two sided) or smaller was considered statistically significant.

To quantitatively assess similarity in spatial distribution patterns of the electromechanical, indentation, and thickness mappings between the joints of the same donor (within-donor) versus different donors (between-donor), parametric correlation analysis (Pearson’s r and Spearman’s ρ) was performed. Mappings of all right and left (mirrored) articular surfaces were considered. Correlation coefficients measure the similarity in shape (spatial distribution patterns) only, regardless of differences in scale.29–31 Under the assumption that patterns in the distribution of cartilage’s properties should be more similar within-donor than between-donor, it was assumed that finding higher within-donor correlation coefficients would be an indication of mapping characterizations performance.

RESULTS

QP and IM Correlate With Osteochondral Core Assessments

A Pearson’s correlation analysis revealed meaningful correlations between the QP and IM with mechanical properties in UC. Normal cartilage is predicted to show low QP and high IM values. Effectively, a lower QP and a higher IM correlated strongly with a higher $E_f$ ($r = -0.69, p < 0.0001$; $r = 0.70, p < 0.0001$, respectively), moderately with a higher $E_m$ ($r = -0.30, p = 0.005$; $r = -0.49, p < 0.0001$, respectively), and lower permeability log($k$) ($r = 0.56, p < 0.0001$; $r = -0.47, p = 0.0001$, respectively). For tibial plateaus, QP and IM correlated moderately with cartilage thickness ($r = 0.40, p = 0.001$; $r = -0.55, p = 0.0002$, respectively) and tibial plateau cartilage varied from 1.46 to 3.85 mm thick. For distal femurs, a moderate correlation was obtained between QP and thickness ($r = 0.43, p = 0.02$), but no correlation was obtained between IM and thickness ($r = -0.13, p = 0.54$). Distal femur cartilage varied from 1.59 to 3.39 mm thick. Safranin-O/Fast Green stained sections showed evidence of GAG depletion in areas with higher QP and decreased IM and consistent with this finding (Fig. 2), the QP and IM correlated moderately but significantly with the Mankin score which reflects cartilage degeneration ($r = 0.42, p < 0.0001$; $r = -0.38, p < 0.0001$, respectively). Weak to no correlations were found between QP or IM and biochemical parameters. Firstly, altogether these data showed that QP and IM values correlate or not as expected with the other current techniques to assess cartilage degeneration used in this study. Thus, further analysis on the sensitivity of both techniques could be conducted.

Mixed Effects Model Analysis

A multiple regression model (mixed effect) was used in order to examine whether individual osteochondral core assessments could distinguish between regions I, II (i.e., the area surrounding region I), and III (Fig. 3). A difference between regions I and III could be revealed by the histological Mankin score ($p = 0.0003$), the QP ($p < 0.0001$), the IM ($p < 0.0001$), the $E_f$ ($p < 0.0001$), the logarithm of permeability ($p = 0.0134$), and the collagen per wet and dry weight ($p < 0.02$ and $p < 0.004$, respectively). In region II analyses, it was defined as a 5 or a 10 mm area circumferentially adjacent to region I (Fig. 1). The analysis revealed similar results for both region II areas. Between regions I and II (surrounding 5 mm), only the QP (13.7 vs. 10.7 AU, $p = 0.0001$), IM (2.5 vs. 4.4 MPa, $p = 0.0003$) and collagen per dry weight (0.33 vs. 0.29 mg/mg, $p = 0.0424$) had a significantly different adjusted mean. However, when taking the surrounding 10 mm, the Mankin score, $E_f$ and logarithm of permeability could also reveal significant differences between regions I and II (3.4 vs. 2.5, $p = 0.0406$, 9.1 vs. 17.1 MPa, $p = 0.004$, and $-2.3$ vs. $-2.9, p = 0.0448$, respectively). Between regions II (surrounding 5 mm) and III, only the QP (10.7 vs. 8.4 AU, $p < 0.0001$), IM (4.4 vs. 6.2 MPa, $p < 0.0001$), and $E_f$ (13.7 vs. 19.6 MPa, $p < 0.04$) differentiated both macroscopically visually normal regions. However, when taking the surrounding 10 mm, the $E_f$ could not reveal a significant difference between regions II and III (17.1 vs. 17.2 MPa, $p = 0.62$). All other characterizations, such as permeability, water content, GAG content, and cells content per wet and dry weight, did not show any significant differences between those three regions. The differentiation between regions II and III is critical, since both regions are macroscopically normal while region II surrounds lesional cartilage (i.e., early degeneration) where only QP and IM could differentiate significantly region II from region III.

Sensitivity Analysis

An effect size analysis was performed to investigate the sensitivity of different assessments for distinguishing between regions I, II, and III (Fig. 4). The QP, the Mankin score, the IM, thickness, $E_f$, and collagen per wet and dry weight produced an effect size equal or higher than 0.5 for the detection of all pairwise regions: I vs. II, I vs. III (17.2 MPa, $p = 0.62$). All other characterizations, such as permeability, water content, GAG content, and cells content per wet and dry weight, did not show any significant differences between those three regions. The following parameters had an effect size score lower than 0.5 for the detection of all pairwise regions: $E_m$, permeability, water content, GAG content, and cells content per wet and dry weight. These results are consistent with the mixed effects model comparing regions I and III, where only the Mankin score, the QP, the IM, the $E_f$, and collagen per wet and dry weight’s means were significantly different. Between regions I and II and regions II and III, results from the mixed
model and effect size were consistent where the QP, IM, and $E_f$ were significantly different between regions.

**QP, IM, and Thickness Mappings**

Mappings of the QP (Fig. 5a), IM (Fig. 5b), and thickness (Fig. 5c) were obtained for all pairs of articular surfaces. Abnormal cartilage may be thicker due to increased water content or thinner due to tissue loss. The thickness patterns were similar in neighboring tissues and abnormal cartilage was delimited by a red solid line (Fig. 5c) in the distal femurs. Most importantly, these same degenerated regions had high QP (greater than 10 AU, yellow-red regions in Fig. 5a) and low IM (between 0.2 and 3 MPa, yellow-red regions in Fig. 5b) compared to normal regions (between 4 and 10 AU, blue-green regions in Fig. 5a; between 3 and 20 MPa, blue-green regions in Fig. 5b). QP and IM mappings were, therefore, consistent with the macroscopic assessment of abnormal cartilage and in addition revealed that degradation patterns often extended beyond the macroscopically visible lesion boundaries (Fig. 5a and b).

**Bilaterally Similar Distribution Patterns Revealed by Mappings**

Similar distribution patterns were seen when comparing the right and left characterization maps of each donor (Fig. 5, black arrowheads). A quantitative analysis was conducted to compare the performance of the histological Mankin score, thickness, electromechanical QP, instantaneous modulus, and unconfined compression parameters (fibril modulus, matrix modulus, and permeability) in distinguishing between regions I, II, and III. The number of cores in each region for each characterization is shown above each bar. Region II is the surrounding 5 mm around region I. $^* p < 0.05$, $^*^* p < 0.01$, and $^*^*^* p < 0.001$. Bar graph displays adjusted Least Squares Means and Standard Error outputs from the mixed effects model. The legend only shows a representative example of the three regions of one distal femur.
assessment of this within-donor similarity was performed and results are shown in Figure 6. To assess the similarity of the spatial distribution pattern of bilateral knee joints, Pearson’s $r$ and Spearman’s $\rho$ were both calculated. Both parametric analyses revealed similar conclusions. However, Pearson’s $r$ correlation was higher in all cases, suggesting variable responses were reasonably linear (Fig. 6). Moderate correlations were found within-donor for the QP and the IM in distal femurs and tibial plateaus. These moderate correlations within-donor constitutes initial evidence of the hypothesis that the spatial distributions within-donor are similar in terms of shape (Fig. 6), and potentially, in terms of scale (Fig. 5).

**DISCUSSION**

The purpose of this study was to investigate the performance of two recent techniques, one electromechanical and the other mechanical, to distinguish between cartilage regions where degeneration is visible, where early or mild degeneration is suspected (adjacent to visibly degenerated regions) and where no degeneration is suspected (far away from visibly degenerated regions). Meaningful correlations were observed between QP and IM and well-established histological, mechanical, and biochemical assessments (Fig. 2). A mixed effects model and effect size analysis showed that these two parameters were more robust in distinguishing early degenerated cartilage compared to
the other well-established methods (Figs. 3 and 4). The QP and IM mappings were consistent with the macroscopic visual assessment of abnormal cartilage, and they revealed an extended degradation region compared to what was seen visually (Fig. 5). Lastly, these recently developed techniques revealed moderate bilateral similarity of the within-donor spatial distribution (Fig. 6).

All results in this study clearly demonstrated the ability of the arthroscopic probe and the automated indentation technique to distinguish between visually degenerated cartilage, early degeneration (not visible), and normal cartilage. Our results indicated that only the QP and IM were sensitive enough to distinguish early cartilage degeneration that appears visually normal but are adjacent to a defect (Figs. 3 and 4). Indeed, these observations are consistent with previously published studies, where the sensitivity of electromechanics and mechanics to detect early alterations in cartilage has been demonstrated. In particular, the current study is the first to show the superior sensitivity of both quantitative techniques on human cadaveric articular surfaces of naturally degraded cartilage surrounding focal defects compared to semi-quantitative histological Mankin scores which is considered to reflect well the overall condition of the cartilage. Indeed, studies have shown that early-stage OA processes occur without macroscopic changes in the cartilage morphology. Our study also shows that visual and thickness assessments are much less sensitive to distinguish early degenerated cartilage, similarly to many previous studies, than electromechanical and mechanical assessments where the functional and structural properties of cartilage play important roles.

The current study also reveals similar correlations of the QP with histological scores and mechanical parameters in human tibial plateaus and distal femurs as reported in a prior study. In addition, there was a positive correlation between QP and cartilage thickness in tibial plateaus, which could be related to more important topographical variations present on this articular surface compared to distal femurs. It shall be noted that a different indentation speed was used for both articular surfaces. However, based on a previous work, the cartilage seems to attain an elastic regime near 500 μm/s. Albeit, this difference may not have had an important impact on the outcome measurement. The IM, obtained from the automated indentation technique, also correlated with the histological Mankin score and UC parameters as expected. Indeed, both mechanical measurements (IM and UC parameters) are from different test configurations where the cartilage response should reflect the structure and integrity of the collagen network. Weak to no correlations were observed between both electromechanics or mechanics, and biochemical parameters, since we believe that subtle changes occurring in early stages mostly affect the structure/integrity of the macromolecular framework rather than biochemical content.

The strength of these two techniques relies on their quantitative assessment of cartilage degeneration obtained non-destructively and relatively rapidly. These techniques offer many advantages compared to well-established methods of cartilage assessments by characterizing the entire surface non-destructively which could be appreciable in a cartilage repair or in a drug-treatment study. Indeed, both techniques could help assess the surrounding cartilage, while other assessments only provide information at specific locations since evaluations are only performed on harvested locations. In addition, in contrast to what is usually required for simple mechanical tests (i.e.,
individual sample harvesting, visual orientation of the sample surface perpendicularly to compression axis, sample preservation causing possible mechanical alteration), both QP and IM techniques do not require such sample handleings which are costly and time-consuming. Moreover, the role of the camera-registration system accompanying both systems is crucial since it registers the location of test measurements which in the case of the electromechanical probe allows for real-time positioning and, in the case of the automated indentation technique, allows for mechanically controlled positioning over the surface. For clinical evaluation of cartilage, the electromechanical probe offers a major advantage over the automated indentation technique since it can be used as an arthroscopic tool and has already shown reliability in assessing the quality of cartilage and user-independence.\textsuperscript{15,16,41-43}

Nonetheless, the automated indentation technique has the advantage of being used on animals as small as rabbit or mice for in vitro studies.\textsuperscript{44}

A limitation imposed by our study design is that our sensitivity analysis was based on macroscopic visual assessment (assumed comparable to the arthroscopic assessment) for delimiting all regions. Many studies have shown that even if arthroscopic assessment (visual and probing) remains useful for OA diagnosis, its outcome remains subjective and limited to the surface of the articular cartilage.\textsuperscript{10} Using such a qualitative assessment to define regions of cartilage degradation has probably created errors in the region assignment, which could have reduced the performance of more reliable characterization techniques. Region II was defined as either the surrounding 5 or 10 mm. The larger the surrounding distance, the more cores far from the lesions (not suspected of degeneration) are included in region II. This could be observed for the fibril modulus, since by taking the surrounding 10 mm, there was no significant difference between regions II and III contrary to the surrounding 5 mm. However, the inclusion of more samples within each region could increase power and lead to more significant differences between regions II and III for the fibril modulus. In particular, this study has compared absolute measurements (e.g., QP, IM, thickness) to relative diagnostic values (e.g., histological Mankin score). Hypothetically, if the cartilage samples in this study were perfectly normal, the Mankin score would be 0 everywhere, whereas the QP and the IM would still reveal a spatial distribution and this should be considered while interpreting results such as those presented in Figure 4. Using average normal characterization distributions as reference values, our group is currently in the process of interpreting and transforming these measurements into diagnostic criteria. This transition is essential to open the way for the electromechanical arthroscopic probe to clinical applications. Another limitation is the relatively small number of articular surfaces used covering only the very early-stage of OA progression. Even if all pairs of articular surfaces showed a left-right moderate similarity in the distribution pattern of both electromechanical and mechanical parameters, where this symmetry was previously only observed at late-stage OA,\textsuperscript{45} there is a high variability of the data both within- and between-donor. These primary observations are initial evidence that the hypothesis on within-donor similarity may be correct. However, the inclusion of more donors would support investigation on the timescale involvement of this symmetrical process for clinical purposes of bilateral knee osteoarthritis. Nonetheless, there are so many variables and measurements that multiplicity issue should be considered. Another limitation is related to the automated indentation technique where the Hayes’ model\textsuperscript{28} was used to calculate the elastic modulus (IM) in spherical indentation using the transient section of the load/displacement curve. Data could have been fit to more appropriate models of articular cartilage mechanics, for example, the fibril-reinforced poroelastic model,\textsuperscript{25} where the permeability, the matrix, and fibril modulus can be fitted from the stress relaxation portion of the curve.

This study is the first to report the ability of an electromechanical probe and automated indentation technique to identify early signs of alterations of articular cartilage. These new techniques have not only been found to be more sensitive than well-established techniques that require harvesting a cartilage explants, but are less time-consuming, and reveal interesting patterns of bilateral similarity for normal articular surfaces and early OA sites. These mapping techniques provide new avenues for innovative study designs (e.g., cartilage repair\textsuperscript{21,46}, where entire articular surfaces could be rapidly and non-destructively assessed, either ex vivo (benchtop electromechanical probe or multiaxial mechanical tester) or in vivo, using the arthroscopic electromechanical probe.

**AUTHORS’ CONTRIBUTIONS**

SS: Research design, acquisition, analysis and interpretation of data, drafting, and revision of the paper. AC: Research design, acquisition of histological and biochemical data, and critical revision of the article. MG: Research design, interpretation of the data, and critical revision of the article. EQ: Research design, interpretation of the data, and critical revision of the article. PL: Provided clinical input and critical revision of the article. MDB: Research design, analysis and interpretation of data, statistical expertise, and critical revision of the article. All authors have read and approved the final submitted manuscript.

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REFERENCES


