Streaming Potential-Based Arthroscopic Device is Sensitive to Cartilage Changes Immediately Post-Impact in an Equine Cartilage Injury Model

Models of post-traumatic osteoarthritis where early degenerative changes can be monitored are valuable for assessing potential therapeutic strategies. Current methods for evaluating cartilage mechanical properties may not capture the low-grade cartilage changes expected at these earlier time points following injury. In this study, an explant model of cartilage injury was used to determine whether streaming potential measurements by manual indentation could detect cartilage changes immediately following mechanical impact and to compare their sensitivity to biomechanical tests. Impacts were delivered ex vivo, at one of three stress levels, to specific positions on isolated adult equine trochlea. Cartilage properties were assessed by streaming potential measurements, made pre- and post-impact using a commercially available arthroscopic device, and by stress relaxation tests in unconfined compression geometry of isolated cartilage disks, providing the streaming potential integral (SPI), fibril modulus (Ef), matrix modulus (Em), and permeability (k). Histological sections were stained with Safranin-O and adjacent unstained sections examined in polarized light microscopy. Impacts were low, 17.3 ± 2.7 MPa (n = 15), medium, 27.8 ± 8.5 MPa (n = 13), or high, 48.7 ± 12.1 MPa (n = 16), and delivered using a custom-built spring-loaded device with a rise time of approximately 1 ms. SPI was significantly reduced after medium (p = 0.006) and high (p < 0.001) impacts. Ef, representing collagen network stiffness, was significantly reduced in high impact samples only (p < 0.001 lateral trochlea, p = 0.042 medial trochlea), where permeability also increased (p = 0.003 lateral trochlea, p = 0.007 medial trochlea). Significant (p < 0.05, n = 68) moderate to strong correlations between SPI and Ef (r = 0.857), Em (r = 0.493), log(k) (r = −0.484), and cartilage thickness (r = −0.804) were detected. Effect sizes were higher for SPI than Ef, Em, and k, indicating greater sensitivity of electromechanical measurements to impact injury compared to purely biomechanical parameters. Histological changes due to impact were limited to the presence of superficial zone damage which increased with impact stress. Non-destructive streaming potential measurements were more sensitive to impact-related articular cartilage changes than biomechanical assessment of isolated cartilage using stress relaxation tests in unconfined compression geometry. Correlations between electromechanical and biomechanical methods further support the relationship between non-destructive electromechanical measurements and intrinsic cartilage properties. [DOI: 10.1115/1.4004230]

Keywords: streaming potentials, cartilage biomechanics, impact injury, post-traumatic osteoarthritis, animal model

1 Introduction

Articular cartilage covers the ends of long bones in synovial joints, such as the knee, and is responsible for pain-free, virtually frictionless motion and distributing applied loads to underlying bone. It has a complex extracellular matrix (ECM) mainly composed of large, hydrated proteoglycan molecules trapped in a highly organized fibrillar collagen network. Under compression, interstitial fluid associated with the proteoglycan matrix is pressurized, placing the collagen network in tension, allowing for high dynamic stiffness and load-bearing [1–4]. The collagen network provides the structural architecture of cartilage, immobilizes proteoglycan, which resist fluid flow, and thereby resists tissue compression and expansion to provide effective load bearing.

Cartilage also exhibits electromechanical behavior due to negatively charged sulfate and carboxyl groups on proteoglycan that are bathed in extracellular fluid bearing a net positive charge due to Donnan equilibrium [5–8]. During compression, the excess positive mobile sodium ions are displaced relative to the fixed proteoglycan, generating streaming potentials. Streaming potentials reflect the structure and composition of cartilage and are known to be sensitive to enzymatic and cytokine induced degradation [9–14].

The stratified and functionally important ECM of articular cartilage forms during normal post-natal development processes and is maintained in adulthood by a sparse population of resident chondrocytes. Consequently, articular cartilage has limited intrinsic repair capacity. In osteoarthritis (OA), degradative processes...
overwhelm the ability of chondrocytes to maintain the ECM [15]. Arthritic cartilage is mechanically weak, due to the fragmented collagen network and proteoglycan depletion, and abnormally low streaming potentials are generated during compression. OA that develops following joint injury or trauma is described as post-traumatic osteoarthritis (PTOA), and studies suggest that 50% of these patients will develop OA 5–15 years after the initial mechanical insult [16]. Because the initiating event in PTOA is known, a unique opportunity exists during the immediate and acute stages, when degenerative and remodeling processes are elevated, where therapeutic intervention administered to moderate disease progression may have the greatest preventative potential [15–17].

Evaluating potential therapeutic strategies requires animal models of PTOA where early degenerative changes can be monitored. Impact models, where the location and severity of damage inflicted on the joint surface are controlled, are suited to this purpose [17]. Previous studies have delivered impacts using drop-tower devices, pendulums, or free flight masses, which are capable of producing high strain rates [17] with time to peak loads less than 30 ms [18]. Explant impact studies have documented chondrocyte apoptosis and ECM fissuring and depletion as a result of impact loading [19–23].

In vivo studies are often performed in smaller animals like rabbits and dogs [17] where impact is delivered to closed joints [24] or directly to the cartilage surface via a surgical incision [25–27]. In vivo models generally have less control over the delivery of the mechanical insult but provide the appropriate physiological environment for studying acute and chronic degenerative effects of impact, such as upregulation of matrix-degrading enzymes, subchondral bone changes, and altered cartilage biomechanical properties, including stiffness and hydraulic permeability [24,28–32].

Equine models of OA are advantageous because the comparatively large stifle (knee) joint affords arthroscopic access to joint surfaces including the condyles and distal trochlea. Bolam et al. [28] reported irreversible cartilage degradation at 3 months, which continued to progress up to 6 months, following localized impact of 60 MPa in equine medial femoral condyles. At 3 months, degenerative changes included loss of sulfated glycosaminoglycans, superficial zone fissuring, and mild synovitis. By 6 months, more overt fibrillation, cartilage delamination, and lesions on opposing joint surfaces were noted. Studying earlier time points in this model, where a trajectory toward OA has been established, could provide the desired model system of early PTOA where therapeutic strategies could be tested [17]. However, current methods for evaluating cartilage mechanical properties may be inadequate for measuring the low-grade cartilage changes expected at these earlier time points. The sensitivity of the streaming potential method to degeneration [9,12,14] makes it a promising candidate in this regard. A commercially available arthroscopic device non-destructively measures the electric potentials generated during cartilage compression and computes a quantitative parameter, the streaming potential integral (SPI), reflecting cartilage structure, composition, and function.

In the present study, the ability of streaming potentials to detect cartilage changes immediately following localized impacts at three distinct stress levels was assessed in vitro. We hypothesized that streaming potentials could distinguish between impact levels and that they were more sensitive than biomechanical tests.

2 Methods

2.1 Sample Preparation and Experimental Design. Both stifle (knee) joints from a 4 year old Standardbred horse with no pre-existing joint pathology were collected within 1 h of sacrifice and stored at 4°C. The lengthy experimental protocol necessitated testing the right stifle 5 days after the left, and it remained refrigerated with a closed joint capsule and intact surrounding musculature to limit degenerative changes. The trochlea were isolated from each stifle joint, and 36 arthroscopically accessible sites per trochlea were identified on the distal two-thirds of the joint surface (Fig. 1). Sites were spaced approximately 3 mm apart. Twelve sites were controls, and the remaining 24 sites assigned to one of three levels of impact. The placement of impact and control sites accommodated the normal variability in cartilage properties observed on this joint surface [33] and seen during a pilot study.

2.2 Electromechanical Testing. The Arthro-BST, a handheld arthroscopic device for cartilage assessment (Biomomentum Inc., Laval, Canada), was used to non-destructively measure electromechanical properties of articular cartilage during indentation. Streaming potentials were measured by an array of 37 gold micro-electrodes equally distributed on the hemispherical tip (radius of curvature = 3.05 mm) of the device. Associated instrumentation and software captured the streaming potential distribution generated on the spherically shaped tip during a light cartilage indentation. A quantitative parameter, the streaming potential integral (SPI, mV·mm·sec⁻¹), was calculated at a standardized amplitude of compression of 150 μm determined by electrode-tissue contact signals analyzed over time.

Two users measured each site three times for a total of six electromechanical measurements per site. This was done prior to impact and repeated following impact. A digital camera and LABVIEW software assisted users in identifying measurement sites and minimizing error due to repeated positioning of the Arthro-BST device at each predefined location. The joint surface was immersed in PBS solution for approximately 30 min prior to beginning electromechanical measurements.

2.3 Impact Delivery. Impacts, at one of three stress levels, were delivered using a custom-built impactor device, consisting of a spring-loaded shaft and sterilizable, 6.45 mm diameter plane-ended tip with rounded edges, designed for both in vitro and in vivo applications.
vivo studies [28]. Impacts were delivered manually by aligning the device so that the tip was perpendicular to the articular cartilage surface, then compressing the spring and releasing it to transfer the stored potential energy to the articular surface at rise times of approximately 1 ms. Spring compression was standardized by marking the shaft of the impactor at levels known to produce the desired impact stresses, which were established during a pilot study. The trochlea was secured mechanically, and the cartilage surface covered with saline soaked kimwipes, when not being impacted, to prevent dehydration.

Impact stresses were derived from measurements made with a calibrated piezoelectric force transducer (Model 218C, PCB Piezotronics, Depew, NY, USA) placed in-line with the impactor tip and connected via a charge amplifier (Model 421A11, PCB Piezotronics, Depew, NY, USA) to a data acquisition card (Model USB6009, National Instruments) and LabVIEW software. The transducer signal was sampled at 48 kHz, and impact stress calculated by normalizing peak force to the cross-sectional area of the impactor tip, 32.7 mm².

Immediately following impacts, India ink (BD, Franklin Lakes, NJ) was applied to the joint surface, and electromechanical measurements were repeated.

### 2.4 Unconfined Compression Testing
Osteochondral cores were isolated from all sites (n = 72 for both trochlea) using a 3.5 mm diameter mosaic arthroplasty punch (Acufex, Smith and Nephew, Andover, MA) and stored in individual humid chambers at 4 °C until biomechanical testing in unconfined compression geometry on one of two Mach-1 Micromechanical Testers (Biomeomentum Inc., Laval, Canada).

Immediately prior to testing, cartilage was separated from the underlying bone, re-punched to 3 mm diameter, and equilibrated in PBS for 15 min. Cartilage disk thickness was measured, at five locations per disk, with an upright digital micrometer (Mitutoyo, Kawasaki, Japan) and used to calculate test parameters. An initial pre-compression was applied until the top of the cartilage disk was parallel with the bottom. Then each disk was subjected to five stress relaxation ramps of 2% strain applied at a rate of 0.4% strain per second. Between ramps, relaxation was permitted until the load decay was 0.01 g/min (0.23 Pa/s for 3 mm diameter cartilage disks). This criterion for relaxation represents a trade off between complete relaxation, where ideally no load change would be observed, and consideration for the time required to test each cartilage disk. Internal studies indicated that using a lower relaxation rate would add significantly to testing time while producing a nominal decrease in equilibrium load, representing less than approximately 5% of the equilibrium load obtained using 0.01 g/min. The fibril-network-reinforced biphasic model was fit to the data to obtain fibril modulus (Ef), matrix modulus (Em) and hydraulic permeability (k) [34–36].

Outliers were defined as cartilage disks with differences in thickness exceeding 20% or sites that exhibited different cartilage characteristics compared to the majority of sites. Using these criteria, four outliers were identified during biomechanical data analysis.

### 2.5 Histology
Following biomechanical testing, cartilage disks were fixed in a solution of 2.5% cetylpyridinium chloride (CPC), 4% paraformaldehyde, 0.1 M sodium cacodylate, at room temperature. The inclusion of CPC prevents the loss of sulfated glycosaminoglycans to the fixation media, which can occur especially in impacted samples [37]. Cartilage disks were fixed for a minimum of 1 week, paraffin embedded, and sectioned at 5 μm.

Sections were stained with Safranin-O/Fast Green/iron hematoxylin and adjacent sections were mounted unstained for viewing in polarized light microscopy (PLM).

### 2.6 Statistical Analysis
Paired t-tests were used to compare pre- and post-impact SPI. Sensitivity testing was performed by running an equivalent non-parametric test, the Wilcoxon matched pairs test, for comparison. A general linear model one-way ANOVA followed by Fisher’s LSD post hoc tests were used to compare biomechanical parameters at impacted versus control sites. Effect size (difference between control and impact means divided by pooled standard deviation) was calculated between each impact group and controls in order to provide a sensitivity of each measured parameter to impact. Correlations between SPI and biomechanical properties were also explored. Inter- and intra-user reliability of electromechanical measurements were obtained using intraclass correlation coefficients (ICC) for agreement, assessed simultaneously using a repeated measures design [38].

Statistical analyses were performed in STATISTICA v.9 (StatSoft Inc., Tulsa, OK) except ICCs, which were calculated using a custom-built LaVIEW function (LaVIEW v.8.6, National Instruments, Austin TX, USA) validated against results obtained with SPSS v.9 (SPSS Inc. Chicago, IL, USA).

### 3 Results

#### 3.1 Impacts
Measured average impact stress delivered to the trochlear surfaces were either low, 17.3 ± 2.7 MPa (n = 15), medium, 27.8 ± 8.5 MPa (n = 15), or high, 48.7 ± 12.1 MPa (n = 16).

#### 3.2 Electromechanical Measurements
SPI decreased as a function of impact stress on both the medial and lateral trochlear surfaces (Fig. 2). When compared to pre-impact values, paired t-tests detected significantly reduced SPI following high (p < 0.001, n = 16) and medium (p = 0.006, n = 15) impacts but not low impact (p = 0.544, n = 16). Considering the medial and lateral facets separately revealed that while high impacts significantly reduced SPI on both joint surfaces, medium impacts led to a significant reduction on the medial trochlea (p = 0.032, n = 7), and a trend toward a reduction on the lateral trochlea (p = 0.103, n = 8).

Results obtained from an equivalent non-parametric test, the Wilcoxon matched pairs test, were concurrent with parametric testing and significantly (p < 0.05) reduced SPI was detected in all cases described above, including medium impact on the lateral trochlear facet.

Excellent intra- and inter-user agreement among SPI measurements were determined using ICCs and confirmed the user-
independent nature of this streaming potential method (Table 1). ICCs are the ratio of variance in the sample population to that of the sample population plus measurement variance. Thus ICCs close to 1 imply very low measurement variability relative to sample population variability. The inter-user ICC of 0.861 indicates excellent agreement between users’ measurements, while intra-user (average) describes the reliability of using the average of a single user’s three measurements. Individual intra-user ICCs, 0.898 and 0.917, describe the reliability of using a single measurement from User 1 or User 2, respectively. Due to the high reliability and agreement among SPI measurements, average SPI was calculated from the six measurements (3 per user \times 2 users) and used for all data analysis.

3.3 Biomechanical Parameters. Impact produced dose-dependent changes in biomechanical parameters measured during unconfined compression testing, including diminished \(E_f\) and \(E_m\), and increased \(k\) (Fig. 3). These observations were supported by statistical analysis where \(E_f\), \(E_m\), and \(k\), obtained from model fits of the fifth stress relaxation ramps, were compared by treatment. Compared to non-impacted controls, \(E_f\), representing collagen network stiffness, was reduced by high impact (\(p < 0.001\) lateral, \(p = 0.042\) medial), with a parallel increase in permeability, \(k\) (\(p = 0.003\) lateral, \(p = 0.007\) medial). No statistically significant changes in \(E_m\), representing proteoglycan matrix stiffness, were detected. Additionally, statistically significant differences were detected between low or medium impact groups when compared to high impact, including higher \(E_f\) on the lateral trochlea (\(p = 0.042\) low impact, \(p = 0.035\) medium impact), and lower permeability on both lateral (\(p = 0.003\) low impact, \(p = 0.026\) medium impact) and medial (\(p = 0.027\) low impact, \(p = 0.036\) medium impact) joint surfaces. The articular cartilage was thinner (\(p < 0.001\)) on the medial facet, 1.54 \pm 0.18 mm (\(n = 36\)) compared to the lateral, 2.02 \pm 0.17 mm (\(n = 36\)).

3.4 Effect Sizes. Effect sizes for electromechanical and biomechanical parameters all increased as a function of impact stress level (Fig. 4). Among the biomechanical parameters, the largest changes were associated with permeability; however, effect sizes for \(E_f\), \(E_m\), and \(k\) were all lower than the effect sizes for SPI. Higher effect sizes for SPI indicate greater sensitivity to cartilage changes than for purely biomechanical measurements.

3.5 Correlations between SPI and Biomechanical Parameters. Linear regression analysis identified significant correlations between SPI and biomechanical parameters measured during unconfined compression testing (Fig. 5). SPI was positively correlated with both \(E_f\) (\(r = 0.857\), \(p < 0.0001\), \(n = 68\)) and \(E_m\) (\(r = 0.493\), \(p < 0.0001\), \(n = 68\)), and negatively correlated with cartilage thickness (\(r = -0.804\), \(p < 0.0001\), \(n = 68\)). A negative weak correlation with \(k\) (\(r = -0.343\), \(p = 0.004\), \(n = 68\)) was detected.
detected, and this was improved to ($r = -0.484, p < 0.0001$, $n = 68$) when transformed to log(k).

3.6 Histology. India ink uptake allowed cartilage surface disruptions to be visualized immediately after impact [39]. Surface cracking and diffuse staining at high impact sites were observed and contrasted with faint or no staining at low impact sites. Medium impact caused variable changes ranging from faint India ink staining to the appearance of minor cracking.

These macroscopic findings corresponded to light microscopy observations of the articular surface (Figs. 6 and 7) where the majority of high impact sites had large surface tears extending into the transitional zone or upper deep zone. In some medium impact samples, surface roughening and small cracks, which rarely extended beyond the superficial zone, covered the cartilage surface, while in others, the articular surface was relatively smooth with only limited evidence of impact. The surfaces of low impact disks exhibited minimal cracking and occasional superficial zone fissures. Control cartilage disks expectedly had smooth, intact articular surfaces, although some exhibited imperfections similar to the low impact group (Fig. 6).

Polarized light microscopy (PLM) patterns were normal in all groups with birefringent superficial and deep zones separated by a non-birefringent transitional zone (Fig. 6). The majority of control cartilage disks had intense Safranin-O staining with some depletion at the articular surface (Fig. 7). Impacted samples were more likely to exhibit weaker Safranin-O staining, both at the surface and the interterritorial matrix of the deep zone.

4 Discussion
Dose-dependent cartilage changes occurred immediately in response to impact injury delivered to articular surfaces at low (17.3 ± 2.7 MPa), medium (27.8 ± 8.5 MPa), or high (48.7 ± 12.1 MPa) peak stress levels. High, and to a lesser extent medium, impacts caused immediate, measurable damage to the collagen network, detected as a reduction in SPI and Ef, coupled with an increase in $k$, but there was insufficient time for substantial proteoglycan loss to occur, reflected in an invariant Em (Fig. 3) and intensely-stained Safranin-O sections (Fig. 7). The first hypothesis, that streaming potentials could distinguish cartilage changes caused by impacts of different levels, was partially supported. SPI values were reduced as a function of impact stress, and these observations backed by statistically significant differences detected following high ($p < 0.001$) and medium ($p = 0.006$) impacts compared with controls. The second hypothesis, that SPI was more sensitive to these changes than biomechanical testing, was
while electromechanical and biomechanical properties were both reduced as a function of impact stress level, effect sizes for SPI were consistently higher than those for biomechanical parameters at all three impact levels (Fig. 4). The superior sensitivity of SPI measurements to cartilage changes was also demonstrated by statistically significant reductions in SPI after both high and medium impacts, while Ef and k detected cartilage damage in a statistically significant manner only after high impact.

4.1 Increased Sensitivity of SPI to Impact Compared to Unconfined Compression Testing. Controlled impacts delivered to the articular surfaces resulted in varying degrees of cartilage damage (Figs. 6 and 7). Compared with the surface cracking caused by high impact, sites that received medium impacts were roughened with superficial zone cracks observed only in occasional samples. This milder damage was reflected in both SPI and biomechanical properties, although SPI was more sensitive as indicated by the larger effect sizes associated with this method and the statistically significant reduction in SPI, but not biomechanical properties, following medium impact.

Unconfined compression testing, where the lateral edges of the cartilage disk remain free while the top and bottom are in contact with flat, non-porous platens, is advantageous for assessing degradation. The resulting stress relaxation curves can be fit with the fibril-reinforced biphasic model [36,40], where the model parameters specifically reflect the two major components of the ECM [41]. These investigators [41] used enzymatic degradation to selectively diminish either collagen or proteoglycan in cartilage disks and demonstrated that Ef is related to the stiffness of the collagen fiber network and Em to the stiffness of the drained proteoglycan matrix. An increase in permeability was observed in both proteoglycan and collagen depleted cartilage, although a greater increase in fluid flow occurred when the collagen network was disrupted [41]. Drawbacks of this type of biomechanical testing...
include that cartilage disks must be isolated, that the accuracy of the test results are influenced by factors such as cartilage thickness variability, and finally that the lengthy testing protocol requires cartilage disks to be stored, which limits the number of disks that can be tested before storage-related degradation adversely affects tissue properties [42].

Electromechanical measurements reflect cartilage material properties; this is illustrated by the statistically significant correlations identified between SPI and biomechanical parameters (Fig. 5). Similar relationships have previously been observed in our laboratory [43].

Sensitivity of electromechanical measurements to damage caused by blunt impact at medium and high stress levels, averaging 28 and 49 MPa, respectively, was demonstrated in this study (Fig. 2). To the best of our knowledge, this is the first report describing the ability of streaming potentials to detect cartilage damage immediately following mechanical injury. Streaming potentials are produced during cartilage compression when mobile positive ions, contained in the interstitial fluid, are displaced relative to negatively charged proteoglycan molecules immobilized in the collagen fiber network. Impact injury causes collagen network disruption, resulting in greater proteoglycan mobility and consequently, smaller relative displacement of mobile positive ions and lower streaming potentials. Our findings agree with other reports showing that streaming potentials are an effective method for detecting degradative changes induced by cytokines or enzymatic modification [9,12,14]. Frank et al. [12] measured streaming potentials and stiffness simultaneously in bovine cartilage explants where proteoglycans were enzymatically depleted with either chondroitinase-ABC or trypsin. Streaming potentials and stiffness decreased with time following enzyme addition, although streaming potentials were more sensitive. Similarly, Légaré et al. [14] cultured cartilage explants with interleukin-1α, a cytokine that suppresses proteoglycan and collagen synthesis by interacting with a chondrocyte membrane receptor. In this model, changes in streaming potential profiles were detected that corresponded to loss of proteoglycans to the culture media, collagen denaturation, and an increase in tissue compliance.

The arthroscopic device for assessing streaming potentials provided a rapid, non-destructive method where the calculation of the quantitative parameter (SPI) did not depend on the velocity of indentation or device orientation [43]. The high intra- and inter-user ICCs obtained in the present study confirm the user-independent nature of the method [44,45].
4.2 Comparison with Early OA Events. The three impact levels employed in this explant study of cartilage injury corresponded to ranges described by previous investigators in the context of impact models of PTOA. The low stress level, averaging 17 MPa, was targeted because it is at the upper end of the physiological range reported for knee and hip joints, which can reach 18 MPa during daily activities [46,47], and is within the range, 15–20 MPa, suggested as a threshold for chondrocyte apoptosis [23]. Medium impact, averaging 28 MPa, falls within a range, 25–30 MPa, that has been shown to lead to degenerative changes in other animal models [17,21,48]. Finally, the highest stress level, averaging 49 MPa, was selected because it is similar to the 60 MPa used previously in an equine model where osteoarthritic changes occurred [28]. The three targeted stress levels were similar to those used by Borrelli et al. [25] where impacts were delivered using a weighted pendulum in an in vivo rabbit model.

Degradative changes observed after medium and high impacts included increased India ink staining, fissuring, superficial zone damage, and diminished functional properties. These are similar to the ECM alterations described in the literature following impact injury, where initial mechanical disruption of the collagen network results in swelling and leads eventually to secondary loss of proteoglycans [16,19–22,28,29,49–51]. In the present study, impact did not alter Em in a statistically significant manner (Fig. 3), and this finding was expected for the early time point after mechanical injury considered here. Biomechanical testing of cartilage disks occurred over several days following impact and disks were stored at 4 °C in humid chambers immediately post-impact, where proteoglycan loss due to storage is minimal [42]. Staining characteristics in histological sections were similar among groups, although impacted disks were more likely to display proteoglycan depletion at the surface and base of cartilage disks compared to controls.

Notably, our findings differed from Borrelli et al. [25], who reported that rabbit cartilage tolerated peak stresses of 55 MPa with no surface disruptions. This could be due to a longer rise time, approximately 20 ms, compared to our study, where time to peak load was on the order of 1 ms. Higher strain rates cause increased cartilage damage compared to lower strain rates for the same peak stress [20,52]. Additionally, those impacts [25] were delivered in situ, where cartilage is more resilient to loading than in explants.

Although not assessed in this study, it is likely that in low and medium impacted samples, where the cartilage surface remained intact, chondrocytes were damaged or underwent apoptosis [49,53,54]. Surviving chondrocytes respond to impact by releasing precursors to inflammatory cytokines [55], which may be sufficient to initiate degenerative changes that eventually culminate in OA. This occurs at the impacted site and can spread to adjacent cartilage [56].

4.3 Technical Considerations and Limitations. Both stifle joints from a single animal were used in this study providing a total of 72 experimental sites. Site independence was confirmed by post-impact India ink staining, where spaces between impact sites did not accept India ink similar to surrounding normal cartilage. This experimental approach generated a large number of independent sites but did not allow inter-individual variability to be explored. This remains a study limitation because other individuals may respond differently to the absolute impact levels used, although variation is not expected to be large as these impact levels produced similar cartilage changes in a pilot study using trochole obtained from a separate horse.

Four outliers were excluded from biomechanical data analysis. Two samples had large differences in thickness, approximately 30%, among the five thickness measurements made per sample, that caused them to appear stiffer. In this stress relaxation protocol, a pre-compression is applied to ensure that surfaces are parallel before the series of five ramps is applied. When a large difference in thickness occurs, the pre-compression is relatively large, placing the cartilage disk under significant load and compression in advance of the actual test. The majority of cartilage disks had differences in thickness less than 10%. The other two outliers were at the same position on both trochole (Fig. 1). Cartilage characteristics here were inconsistent with the central trochole region, exhibiting high SPI values, small thicknesses, and both were very resistant to impact damage, appearing normal at histology.

Sample geometry may have influenced measured biomechanical properties as alterations to the cartilage surface resulted from impact injury. However, surface cracking in impacted samples was not considered extensive enough to alter the overall geometry of the isolated cartilage disks, and they could be reasonably modeled as 3 mm diameter cylinders. Comparisons among the different treatment groups were therefore possible, although greater deviations from the ideal geometry could have occurred at higher impact levels. Electromechanical properties, obtained by indenting the cartilage surface with the arthroscopic device, are not influenced by sample geometry in the same way as biomechanical tests of isolated cartilage disks, and this may have contributed to reduced variability in SPI measurements compared to biomechanical parameters.

5 Conclusions
Immediate damage to articular cartilage was induced using a custom-built spring-loaded impactor device at three stress levels, ranging from the upper end of physiological stress, 17.3 ± 2.7 MPa, to levels previously shown to cause degenerative cartilage changes, 27.8 ± 8.5 MPa and 48.7 ± 12.1 MPa. Streaming potentials were more sensitive to impact related damage than unconfined compression testing. Parameters from unconfined compression testing were significantly correlated to SPI, further establishing the relationship between non-destructive, electromechanical measurements and intrinsic cartilage properties. This type of impact model applied in vivo and combined with streaming potentials to detect subtle cartilage changes could provide a model system suitable for testing the efficacy of therapeutic agents to mitigate disease progression in the early stages of PTOA. The non-destructive nature of the streaming potential method would make sequential assessment of cartilage over time possible for in vivo models where initial degeneration is focal but that could progress to gradually involve more of the articular surface.

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