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Anisotropic Properties of Articular Cartilage in an Accelerated In Vitro Wear Test

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Abstract

Many material properties of articular cartilage are anisotropic, particularly in the superficial zone where collagen fibers have a preferential direction. However, the anisotropy of cartilage wear had not been previously investigated. The objective of this study was to evaluate the anisotropy of cartilage material behavior in an *in vitro* wear test. The wear and coefficient of friction of bovine condylar cartilage were measured with loading in directions parallel (longitudinal) and orthogonal (transverse) to the collagen fiber orientation at the articular surface. An accelerated cartilage wear test was performed against a T316 stainless-steel plate in a solution of phosphate buffered saline with protease inhibitors. A constant load of 160 N was maintained for 14000 cycles of reciprocal sliding motion at 4 mm/s velocity and a travel distance of 18 mm in each direction. The contact pressure during the wear test was approximately 2 MPa, which is in the range of that reported in the human knee and hip joint. Wear was measured by biochemically quantifying the glycosaminoglycans (GAGs) and collagen that was released from the tissue during the wear test. Collagen damage was evaluated with collagen hybridizing peptide (CHP), while visualization of the tissue composition after the wear test was provided with histologic analysis. Results

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

demonstrated that wear in the transverse direction released about twice as many GAGs than in the longitudinal direction, but that no significant differences were seen in the amount of collagen released from the specimens. Specimens worn in the transverse direction had a higher intensity of CHP stain than those worn in the longitudinal direction, suggesting more collagen damage from wear in the transverse direction. No anisotropy in friction was detected at any point in the wear test. Histologic and CHP images demonstrate that the GAG loss and collagen damage extended through much of the depth of the cartilage tissue, particularly for wear in the transverse direction. These results highlight distinct differences between cartilage wear and the wear of traditional engineering materials, and suggest that further study on cartilage wear is warranted. A potential clinical implication of these results is that orienting osteochondral grafts such that the direction of wear is aligned with the primary fiber direction at the articular surface may optimize the life of the graft.

Keywords

cartilage; wear; friction; anisotropy; collagen

1. Introduction

Articular cartilage is a resilient load-bearing material in diarthrodial joints, and provides excellent friction, lubrication and wear resistance during joint motion. Its unique properties derive from its composition and structure. In normal articular cartilage, water content is 65–80% of its total wet weight (Mankin et al., 1994; Mow and Ratcliffe, 1997). The remaining tissue is divided in two major classes of extracellular matrix, proteoglycans associated with abundant glycosaminoglycans (GAGs) and collagen. Chondrocytes are distributed throughout the tissue. The cartilage microstructure is heterogeneous, and can be divided into four zones through the depth of the tissue: superficial, middle, deep and calcified zones (Mankin et al., 1994; Mow and Ratcliffe, 1997). In the superficial zone, the collagen fibers are oriented parallel to the articular surface, and have a preferential fiber direction that is thought to be aligned with the direction of joint motion (Below et al., 2002).

The oriented fibers lead to anisotropic tensile properties in the superficial zone of cartilage, with higher tensile strength and elastic modulus in the direction aligned with the fibers than perpendicular to them (Huang et al., 2005; Kempson et al., 1973; Roth and Mow, 1980; Woo et al., 1979, 1976). However, it has not been determined how these anisotropic properties affect cartilage wear. The wear response of cartilage in different orientations may be especially important when considering the transplantation of osteochondral autografts and allografts to repair cartilage defects. In this repair strategy, one or more osteochondral grafts are transferred to the defect site from minimally weight-bearing areas in the case of autografts, or from cadaveric donors in the case of allografts. Osteochondral grafts have a success rate that is comparable to other surgical techniques for cartilage repair (Gudas et al., 2013, 2006; Horas et al., 2003; Richter et al., 2016; Ulstein et al., 2014) but that is still far from ideal. In one study by Ulstein et al. that followed patients with osteochondral autografts for an average of 9.8 years, 36% of the patients (5 of 14) underwent additional surgical procedures on their treated knee (Ulstein et al., 2014). The long-term success of these grafts

likely relies on multiple factors, including harvesting technique (Hafke et al., 2016), graft geometry (Ackermann et al., 2019), and defect size (Lynch et al., 2015). A better understanding of cartilage wear properties and their relationship to collagen fiber direction could identify another factor affecting osteochondral graft longevity.

Wear is typically quantified for engineering materials by the loss in mass due to wear. Because cartilage is a hydrated tissue and this measurement is difficult (Ateshian et al., 2005), a variety of wear measures have been reported. One of the first and simplest methods for quantifying cartilage wear was by measuring its loss-of-depth. Radin et al (1982) prepared histologic slides from bovine metatarsophalangeal joints that were subjected wear, and compared cartilage depth to that of the contralateral control. Another method of determining cartilage wear is to measure the size, shape and/or number of wear particles, which has been performed *in vivo* and *in vitro*. Kuster et al. (2002) collected wear particles from the knees of patients by joint lavage. SEM images of the particles were used to determine that wear particles from healthy and osteoarthritic cartilage are different in shape due to the distinct arrangement of collagen fibers in different zones of cartilage. In an *in vitro* test, Oungoulian et al. (2013) slid loaded cartilage plugs against a glass surface for 24 hours and analyzed the particulate size and number in the fluid bath. The authors reported that the number and volume of particulates 0.6 μm to 60 μm in size were a sensitive measure of wear. In the current study, biochemical analysis of the cartilage extracellular matrix that was removed from the tissue was used to quantify the wear in cartilage. Hydroxyproline is an amino acid which is very abundant in collagen but rare in other proteins (Mow et al., 2005; Mow and Ratcliffe, 1997). Its presence in the solution bath of a cartilage wear test is an indicator of collagen loss due to wear (Ateshian et al., 2005; Lipshitz et al., 1975; McGann et al., 2012; Oungoulian et al., 2013; Trevino et al., 2017). Similarly, GAGs in the solution bath can also be quantified biochemically and are a measure of proteoglycan loss due to wear (Ghadially, 1981; Oungoulian et al., 2013; Trevino et al., 2017). These wear measures were chosen because quantification of the release of these two matrix components to the solution bath provides information on the nature of the cartilage matrix degradation with mechanical wear.

The objective of this study was to investigate the anisotropic nature of cartilage in an accelerated *in vitro* wear test. Cartilage wear in the direction of the collagen fibers (longitudinal) and perpendicular to them (transverse) was determined by biochemically quantifying the GAGs and collagen that were released to the hydrating fluid bath during the wear test. The coefficient of friction (COF) throughout the wear test was also measured. Collagen damage from the wear test was evaluated with collagen hybridizing peptide (CHP), which binds to denatured collagen and is conjugated to a fluorophore to allow for visualization of collagen damage (Zitnay et al., 2017). Finally, the distribution of proteoglycans and collagen in the tissue was visualized via histology after the wear test was completed and the optical density of the stains were quantified.

2. Methods

2.1 Specimen preparation and fiber orientation

Bovine stifles joints from approximately 1-year old animals were collected from a local abattoir (This Old Farm, Colfax, IN) and stored at −20°C before further use. Cylindrical osteochondral specimens 9.5 mm in diameter and approximately 18 mm in height were cored from the thawed femoral condyles such that the articular surface was perpendicular to the coring axis. Specimens for this study were harvested from 8 different stifles and were distributed randomly to the longitudinal and transverse groups. Fiber direction at the articular surface was estimated from the cartilage split-lines at the periphery of the specimens (Below et al., 2002). The articular surface was pierced with a pin in 14–16 locations near the edge of the specimen and India ink was applied. The excess India ink was removed with dry gauze and the remaining ink identified the preferential direction of the collagen fibers (Fig. 1A).

The split lines at the periphery of the specimen were used to orient the specimen during the wear test. After the wear test, wear lines were also visible with India ink (Fig. 1B). The final determination of the angle between the fiber orientation and wear direction was based on the split line analysis on the cartilage surface after the wear test was complete. This analysis was done on the entire surface of the cartilage, though only regions where the superficial zone had not been worn off could be used to determine the fiber direction (Fig. 1C). An image of the split lines was taken with a stereomicroscope (Stemi 508, Carl Zeiss, Jena, Germany) equipped with a digital camera (Axiocam ERc 5s, Carl Zeiss), and the average angle between the fibers and the wear direction was determined for each specimen using ImageJ software (NIH).

2.2 Wear and friction

An accelerated cartilage wear test was performed against a T316 stainless-steel plate with a #8 mirror finish using a UMT Tribolab (Fig. 2; Bruker, San Jose, CA). The bone portion of the osteochondral specimen was clamped in the device. Specimens were manually oriented based on a visual assessment of the split-lines at the periphery of the specimen such that the primary direction of the collagen fibers was aligned either with the direction of motion (longitudinal) or perpendicular to this direction (transverse). The wear test was performed in a solution of phosphate buffered saline (PBS) with protease inhibitors (1mM ethylenediaminetetraacetic acid, 5mM benzamadine and 10mM n-ethylmaleimide) at room temperature. Preliminary studies identified a test protocol that caused measurable wear. The specimen was lowered toward the stainless steel plate at a rate of 1 mm/s until a “touch force” of 0.2 N was reached. Then, the specimen was lowered at a rate of 0.1 mm/s to a sustained load of 160 N. This load was maintained for 14000 cycles of reciprocal sliding motion at 4 mm/s velocity and a travel distance of 18 mm in each direction. The wear test took approximately 43 hrs for each specimen and the total wear distance was approximately 504m. The friction and normal forces were collected from a two-axis load cell at a rate of 1 kHz throughout the wear test. Additionally, specimens that were exposed to the reciprocating motion of the wear test without an applied load and those that experienced the

160 N load for the same duration of the wear test without the reciprocating motion served as controls.

To quantify cartilage wear, biochemical assays were used to measure the amount of GAGs and collagen released to the solution bath during the wear test. The solution along with any particles that had been released to the bath during wear were collected at the end of the test and stored at −20°C before further analysis. These samples were lyophilized, re-suspended in papain digest solution and incubated at 60°C overnight. The GAG content of the papain digests was determined with a dimethylmethylene blue (DMMB) assay, as has been previously reported (Bonitsky et al., 2017). Aliquots of the papain digest were hydrolyzed at 100°C for 18 h in concentrated HCl (38%). A chloramine-T assay was performed on the hydrolysates to quantify their hydroxyproline content (Bonitsky et al., 2017; McGann et al., 2015). The collagen content was calculated using a hydroxyproline:collagen ratio of 1:7.69 (Ignat'eva et al., 2007).

The ratio of the friction and normal forces was computed to determine the COF during the ~43 hr duration of the experiment. For this analysis, the COF data during acceleration and deceleration was discarded, and only the COF data during the 4 mm/s velocity was considered. COF values were averaged for each reciprocating cycle (both directions) using Matlab (Mathworks, Natick, MA). The average value from the first reciprocating cycle was considered the initial COF. Because the COF reached equilibrium by 4 hr, the average COF of the last 39 hr was taken as the equilibrium COF.

Wear and friction were analyzed for four different groups. First, all longitudinal and transverse samples (n = 12 for each, or 24 total) were analyzed. To determine whether the anatomic location of the harvest site impacted differences in wear between the longitudinal and transverse directions, cartilage specimens that had been collected from medial (n = 6 to 7 each for longitudinal and transverse) or lateral condyles (n = 5 to 6 each) were analyzed separately. Finally, specimens in which both longitudinal and transverse cartilage specimens had been harvested from the same stifle were analyzed (n = 7 each) to minimize the joint-to-joint variability. For each group, GAG wear, collagen wear, initial COF and equilibrium COF of the cartilage were reported.

2.3 Collagen damage and histology

To better understand the effect of longitudinal and transverse wear on cartilage collagen damage and matrix loss, some wear test specimens from medial condyles were assessed with collagen hybridizing peptide (CHP) and histology. At the end of the wear test, cartilage was removed from the bone with a scalpel and was cut in half perpendicular to the articular surface. One half was incubated with 5 μM CHP conjugated to fluorescein (3Helix, Salt Lake City, UT) in PBS for 1 hr at 4°C, rinsed overnight at 4°C, and was imaged with an inverted confocal microscope (FV1000MPE, Olympus America, Melville, NY). The average fluorescence intensity of the tissue was determined with ImageJ software (n=5 each for longitudinal and transverse). The other half was fixed in paraformaldehyde, embedded in paraffin, and 5 μm sections were stained with Safranin-O/Fast Green or Modified Masson's trichrome to visualize GAG and collagen distribution, respectively. Proteoglycan content of one section per specimen was determined with digital densitometry of the Safranin-O stain

(n = 6, Kiviranta et al., 1985). The standard Trichrome staining protocol was modified to include enzymatic digestion of the proteoglycans with papain before staining, as this provides good correlation between collagen content and optical density of the stain (Rieppo et al., 2019). The collagen content of one section per specimen was determined with the digital densitometry of the trichrome stain (n = 6 each for longitudinal and transverse). Images were taken using a light microscope (Leica DM3000, Wetzlar, Germany) connected to a digital camera (Leica DFC290). Staining intensity was quantified by transforming the red channel in Safranin-O images and the blue channel in Trichrome images to gray scale, and then converting average gray scale values to the tissue to optical density.

2.4 Statistics

Data were tested for normality with the Kolmogrov-Smirnov test. Where necessary, data were evaluated with an equivalent nonparametric test. Quantitative differences between the longitudinal and transverse wear tests were determined with a Student's t-test with the Bonferroni correction for repeated measures where appropriate (Graphpad Prism Software, La Jolla, CA). For the analysis of data where a longitudinal and transverse specimen came from the same stifle, a paired t-test was used. Significance was set at $p < 0.05$. Data are presented as mean ± standard deviation.

3. Results

3.1 Fiber orientation

After the accelerated wear test, loss of the superficial zone was observed in most specimens, primarily in the center of the articular surface. Wear lines in the direction of reciprocating motion were visible. The split lines at the periphery of the specimen did not appear to initiate or exacerbate the wear (Fig. 1B), perhaps due to the inherent slight curvature of the specimens (McGann et al., 2012). To validate the relationship between the direction of motion and the fiber orientation, the average angle between fiber and wear directions was determined from the split line analysis of the remaining articular surface after the wear test. The values were 4.2 ± 12.0° for the longitudinal specimens and 79.8 ± 15.8° for the transverse specimens.

3.2 Wear and friction

The matrix components released to the solution bath during the wear test, including from control specimens with no load or no motion, were quantified. The no-load controls released 1.53 ± 0.64 mg GAGs and 66.0 ± 32.4 μg collagen to the solution bath, while the no-motion controls released 0.51 ± 0.39 mg and 40.4 ± 34.9 μg GAGs and collagen, respectively. Because the no-load controls released more matrix overall, the average amount of GAGs and collagen that were released to the solution bath from the no-load controls were subtracted from the respective wear test values to determine the matrix components released due to wear only. When all wear specimens were considered, the transverse wear test resulted in 1.84 ± 0.92 mg GAGs released from the tissue, while the GAGs released due to wear in the longitudinal direction was significantly less at 0.837 ±0.85 mg (Fig. 3A). Similarly, significantly more GAGs were released in the transverse direction when just specimens from the medial condyle were considered, but there was no significant difference between

longitudinal and transverse directions when the specimens originated from the lateral condyle or when the specimens were harvested from the same bovine stifle (Fig. 3A). The collagen released to the saline bath was 262.4 ±123.0 μg and 207.6 ± 89.6 μg for wear in the longitudinal and transverse directions, respectively, when all samples were considered. No significant differences in collagen released during the wear test were detected in any analysis, including when the anatomic location of the harvest site was taken into consideration or when a paired analysis was performed with specimens taken from the same stifle (Fig. 3B).

For both the longitudinal and transverse wear test, the COF was initially low, and with time, the COF increased, consistent with previous studies (Bonitsky et al., 2017; Forster and Fisher, 1999; Krishnan et al., 2004; Lewis and McCutchen, 1959; McGann et al., 2015; Northwood and Fisher, 2007). After approximately 4 hr the COF reached equilibrium and was nearly constant for the remaining 39 hr for both longitudinal and transverse wear (Fig. 4). For the longitudinal case, the average COF increased from an initial value of 0.00358 ± 0.00059 to an equilibrium value of 0.265 ± 0.033. For transverse case the COF rose from 0.00345 ± 0.00041 to 0.247 ± 0.034. No significant differences were found in the initial and equilibrium COF values between longitudinal and transverse direction for any group (Figs.3C and D).

3.3 Collagen damage and histology

CHP was used to visualize collagen damage due to the wear tests in the longitudinal and transverse directions. Confocal imaging of the cartilage tissue showed greater CHP staining in specimens that had been worn in the transverse direction than in the longitudinal direction, with high intensity staining at the articular surface. The average fluorescence intensity was 12.2 ± 1.8 and 24.1 ± 11.0 for longitudinal and transverse, respectively (Fig. 5).

Histological analysis of the wear test specimens supported the results of GAG and collagen wear. Safranin-O staining indicated that specimens that had been worn in the transverse direction were more depleted of GAGs than those that had been worn in the longitudinal direction, and that the GAGs loss extended through most of the depth of the cartilage (Fig. 6A). The optical density of the Safranin-O staining was 0.509 ± 0.043 for specimens that had been worn in the longitudinal direction, and 0.437 ± 0.061 for the transverse direction. On the other hand, Modified Masson’s trichrome staining of collagen appeared approximately equivalent between longitudinal and transverse specimens (Fig. 6B). The optical density of the trichrome stain was 0.494 ± 0.071 and 0.481 ± 0.054 for wear in the longitudinal and transverse directions, respectively.

4. Discussion

The objective of this study was to investigate the anisotropic nature of cartilage in an accelerated *in vitro* wear test. Based on the amount of GAGs released to the solution bath in the accelerated wear test, cartilage wear was anisotropic, as more GAGs were released from the cartilage during transverse wear than during longitudinal wear (Fig. 3A). Histological analysis supported the wear results, demonstrating that fewer GAGs remained in the

specimens subjected to transverse wear compared to longitudinal wear (Fig 6A). In contrast, there was no difference in collagen released from the cartilage for any of the groups that were analyzed (Fig. 3B), and the collagen content measured via quantification of Trichrome staining was also equivalent between the two groups of specimens (Fig. 6B). Instead, CHP staining varied with the direction of wear. Transverse loading in the wear test tended to cause greater CHP staining than loading in the longitudinal direction (Fig. 5). The increased wear was not due to increased friction forces, as the COF was equivalent in the longitudinal and transverse directions (Fig. 3C–D, Fig. 4). The data indicating anisotropic wear from the accelerated wear test are consistent with cartilage's anisotropic tensile material properties (Huang et al., 2005; Kempson et al., 1973; Roth and Mow, 1980; Woo et al., 1979, 1976).

No significant differences were detected between longitudinal and transverse wear for the amount of collagen released during the wear test, or for initial and equilibrium COF (Fig 3B–D). Although large variation in mechanical properties is inherent in biological tissues, we sought to ensure that anatomic variation or variation between donors was not masking a difference in these properties with wear direction. Osteochondral specimens from the medial and lateral condyles were analyzed separately to partially remove the effect of anatomic location, and longitudinal and transverse specimens that originated from the same stifle were analyzed as pairs to address donor-specific effects. However, none of these analyses revealed differences in collagen released during wear or either COF measurement between the longitudinal or transverse wear directions. On the other hand, differences in GAG loss were sustained for specimens from medial condyles but not from lateral condyles. This may be due to anatomic differences in the structure of the collagen network. The orientation of the collagen fibers in the superficial zone has been associated with mechanical loading *in vivo* (Below et al., 2002), and the medial condyle typically withstands greater contact forces (Mündermann et al., 2008; Zhao et al., 2007). Higher forces in the medial condyle may generate a more uniform fiber orientation at the articular surface, resulting in significant anisotropy in wear and damage.

Although this study is the first to investigate anisotropy in cartilage wear, aspects of the study can be compared to previous reports. In the current study, the amount of GAGs released from the cartilage was greater than collagen released for both wear specimens and unloaded controls (Fig. 3A and B), consistent with the report from Trevino et al. (2017) on the wear of viable cartilage tissue against metal. This group also demonstrated GAG loss through the depth of the tissue in histological sections of the wear specimens, but no apparent loss of collagen, consistent with histological analysis in the current study (Fig. 6). On the other hand, our results differ considerably from Oungoulian et al. (2013), who were not able to biochemically detect the release of GAGs or collagen to the solution bath due to wear. This is may be due to differences in the duration of the wear test, as the previously reported study lasted 24 hours and the current one was nearly twice as long. In the current wear test, very little wear was visible in the first 24 hours.

The main limitation with this study is that, due to practical considerations, the wear test was accelerated compared to physiologic wear. The contact pressure during the wear test was approximately 2 MPa, which is in the range of that reported in the human knee and hip joints (Brand, 2005). However, this physiologic load magnitude was applied constantly for

~43 hr, which is a non-physiologic cartilage load history. *In vivo*, peak load durations are short and then the load releases, allowing for fluid exudation and resorption to occur cyclically. In the current wear test, loading for the duration of the wear test prolonged the fluid exudation, increasing the COF and load transfer to the solid matrix (Krishnan et al., 2004), in order to cause measureable wear in an experimentally viable time period. Another aspect of the wear test that led to accelerated wear was the selection of polished stainless steel for the opposing surface. Cartilage-on-metal wear is significantly higher than cartilage-on-cartilage wear in both *in vitro* (McCann et al., 2008; Trevino et al., 2017) and *in vivo* studies (R.J.H. Custers et al., 2010; R.J.H. Custers et al., 2010). Despite the accelerated nature of the wear test, it generated some of the hallmarks of osteoarthritis, namely tissue loss and collagen damage. In osteoarthritic cartilage, depletion of GAGs has been observed to precede the loss of collagen from the tissue (Otsuki et al., 2008; Pettipher et al., 1989; Rolauffs et al., 2010; Saarakkala et al., 2010). This is consistent with the GAG loss in the middle and deep zones seen in the current wear test (Fig 6A). Additionally, many studies have shown that fibrillation, lesions, and disintegration of the collagen network in the superficial zone occur early in osteoarthritis (Clarke, 1971; Henao-Murillo et al., 2018; Hosseininia et al., 2013; Hwang et al., 1992; Panula et al., 1998; Saxena et al., 1991), similar to the striated wear lines and tissue loss in the superficial zone that were observed in the wear test (Fig. 1B). Finally, as in the wear test, osteoarthritic cartilage tissue stains more strongly for CHP than healthy tissue in the superficial and midzone of osteoarthritic cartilage, indicating extensive collagen damage and degradation in the diseased tissue (Hwang et al., 2017). The accelerated wear test, therefore, may be useful to study some aspects of mechanical wear and degradation that are observed in diseased states of cartilage.

No difference was detected in the collagen released during wear in the longitudinal and transverse directions or in the histological analysis of collagen content of the specimens after the wear test. Masson's trichrome stains the intact collagen network blue, but has been reported to stain denatured collagen red (Chvapil et al., 1984). Although no red stain was visible in our histological sections, CHP staining of denatured collagen was observed. The fluorescent CHP marker may be a more sensitive indicator of denatured collagen than the Masson's trichrome histological stain. More abundant CHP stain in transverse wear specimens indicates that the loads from the wear test may have caused additional collagen damage in the transverse direction, but were insufficient to remove collagen from the interconnected network. Increased GAG loss from the same transverse wear test specimens suggests that collagen damage and GAG loss may be connected: either the release of GAGs leads to greater collagen damage, or damage to the collagen network releases GAG-associated proteoglycans that would be otherwise trapped by an intact collagen network. However, the relationship between GAG loss and collagen damage in cartilage tissue, and whether one can cause the other, in either *in vitro* testing or in physiologic disease progression, is unknown. The accelerated wear test may provide a platform for further studies on how these modes of cartilage degradation can influence each other.

Results of the wear test identified some significant differences between cartilage wear and the wear of engineering materials. The wear of engineering materials is often considered a surface phenomenon, as wear results in the removal of material at or near the surface (Chattopadhyay, 2001; Davis, 2001; Mellor, 2006). Because engineering composites

experience surface wear, the composition of their wear debris is assumed proportional to the surface composition. Although the solid cartilage matrix is composed of approximately 70% collagen (Mow and Guo, 2002; Venn and Maroudas, 1977), the wear test resulted in the release of approximately 5 times more GAGs than collagen. This may be because the collagen molecules are tightly bound to one another in a continuous network, while the large, GAG-rich proteoglycans are physically trapped by this network but are not covalently bonded to it for the most part. Additionally, loss of GAGs (Fig 6A) and CHP stain (Fig. 5C) extended through much of the depth of the tissue and were not confined to the surface. The current results suggest that cartilage wear is distinct from that of engineering materials and that further studies are required to better understand the physical mechanism governing cartilage wear.

Results of the study demonstrating anisotropic wear in the accelerated wear test may have implications for the wear of osteochondral grafts. The results suggest that the orientation of osteochondral grafts may affect its wear, and that aligning the collagen fibers at the articular surface with the direction of joint motion may maximize the life of the transplant. Minimizing the wear may also be important to reducing inflammation of the joint after the transplantation of osteochondral grafts, as cartilage wear particles have been shown to induce an inflammatory response in fibroblast-like synoviocytes (Estell et al., 2019). Additionally, the results of this study are important for biomechanical engineers who are performing *in vitro* wear tests; the orientation of the cartilage may need to be taken into account to conduct a consistent wear analysis.

In conclusion, articular cartilage demonstrated anisotropic properties in the accelerated *in vitro* wear test. More GAGs were released from the tissue when the direction of wear was oriented perpendicular to the preferential fiber direction. CHP staining intensity was higher when the wear direction was perpendicular to the fiber direction, suggesting that collagen damage may be higher in this case, but this did not lead to a higher amount of collagen released from the tissue. The anisotropic cartilage wear did not appear to be influenced by the COF, which did not vary with the direction of wear. Cartilage wear was not limited to the articular surface, as the GAG loss and CHP stain extended through the depth of the tissue, particularly when the wear was transverse to the primary fiber direction. These results indicate distinct differences between cartilage wear and the wear of traditional engineering materials, and suggest that further study on cartilage wear is warranted. A potential clinical implication of these results is that orienting osteochondral grafts such that the direction of wear is aligned with the primary fiber direction at the articular surface may optimize the longevity of the graft.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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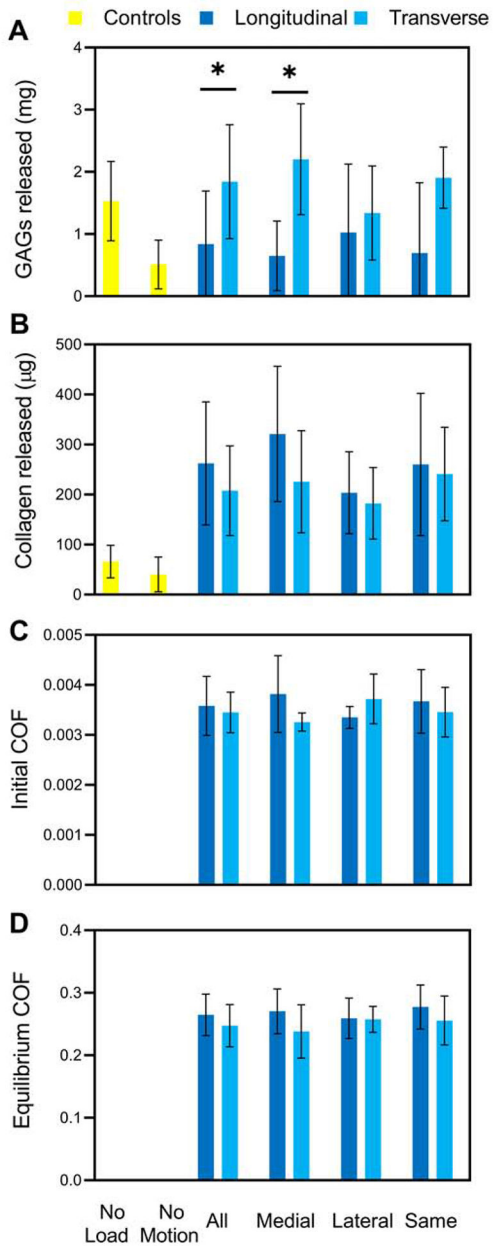

Figure 1. Fiber angle analysis.

A) Split-lines identifying fiber direction at the periphery of the articular surface were used to determine specimen orientation during the wear test. B) Wear lines were visible after the wear test. C) Angle between the fiber and wear directions were determined over the remaining articular cartilage surface. Scale bar A-C: 1 mm. D) Measurement of angle between fiber and wear directions. Scale bar: 100 μm.

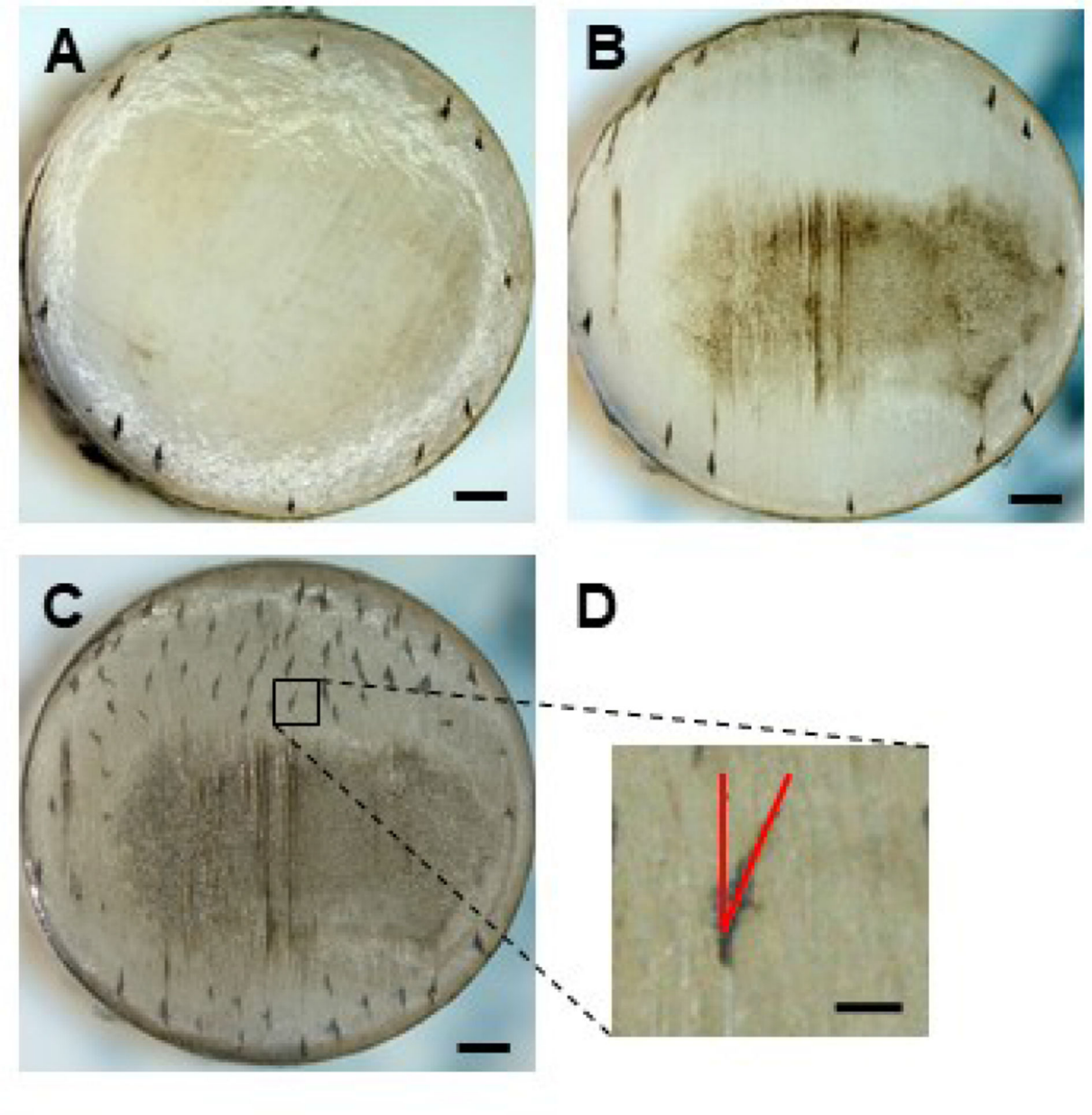

Figure 2. Wear test set-up.

Osteochondral specimens underwent reciprocating motion while loaded against a polished T316 stainless-steel plate. The wear test was performed in PBS containing protease inhibitors. Friction and normal forces were collected from the two-axis load cells and the COF was calculated.

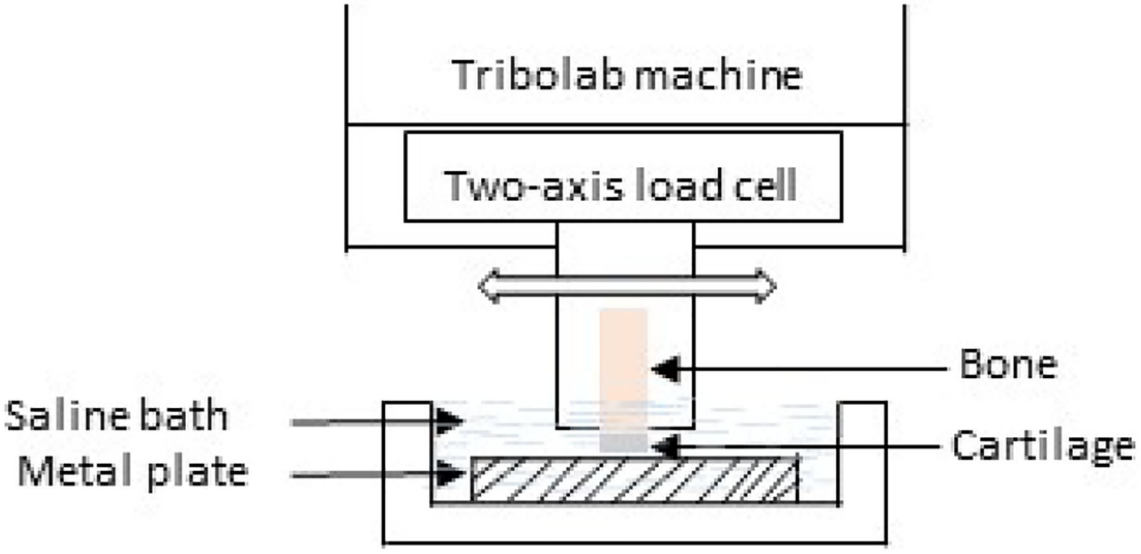

Figure 3. Wear and friction.

Measures of wear and coefficient of friction (COF) in the longitudinal and transverse direction for four different groups, including GAGs and collagen released for no-load controls and no-motion controls. All: All specimens in the study; 2) Medial: Only specimens that originated from the medial condyle; Lateral: Only specimens that originated from the lateral condyle; Same: Only specimens where both longitudinal and transverse specimens were harvested from the same stifle. A) GAGs that were released from the specimens; B) Collagen that was released from the specimens; C) Initial COF; D) Equilibrium COF during the wear test. * indicates $p < 0.05$.

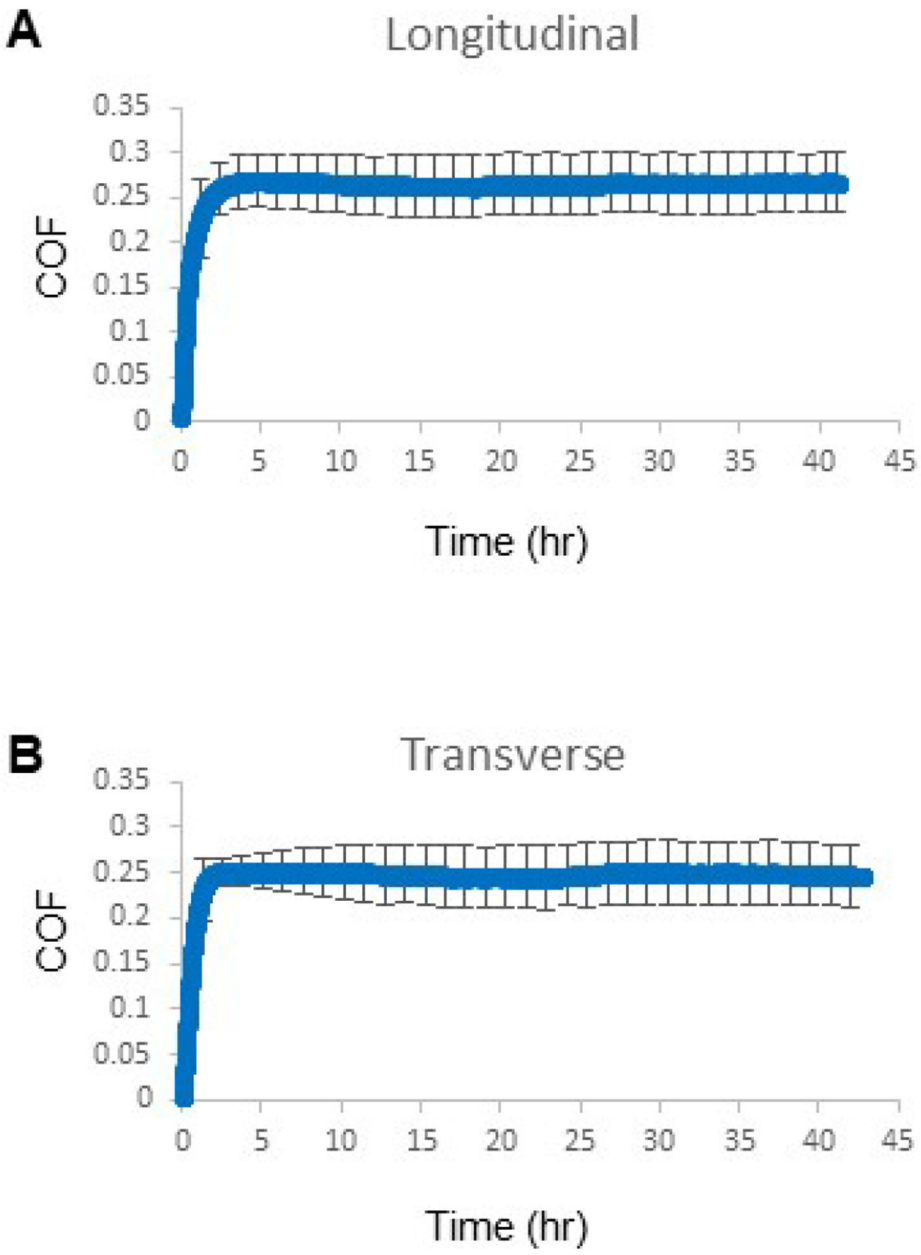

Figure 4. Coefficient of Friction (COF).
The COF over the ~43 hr wear test in the A) longitudinal and B) transverse direction.

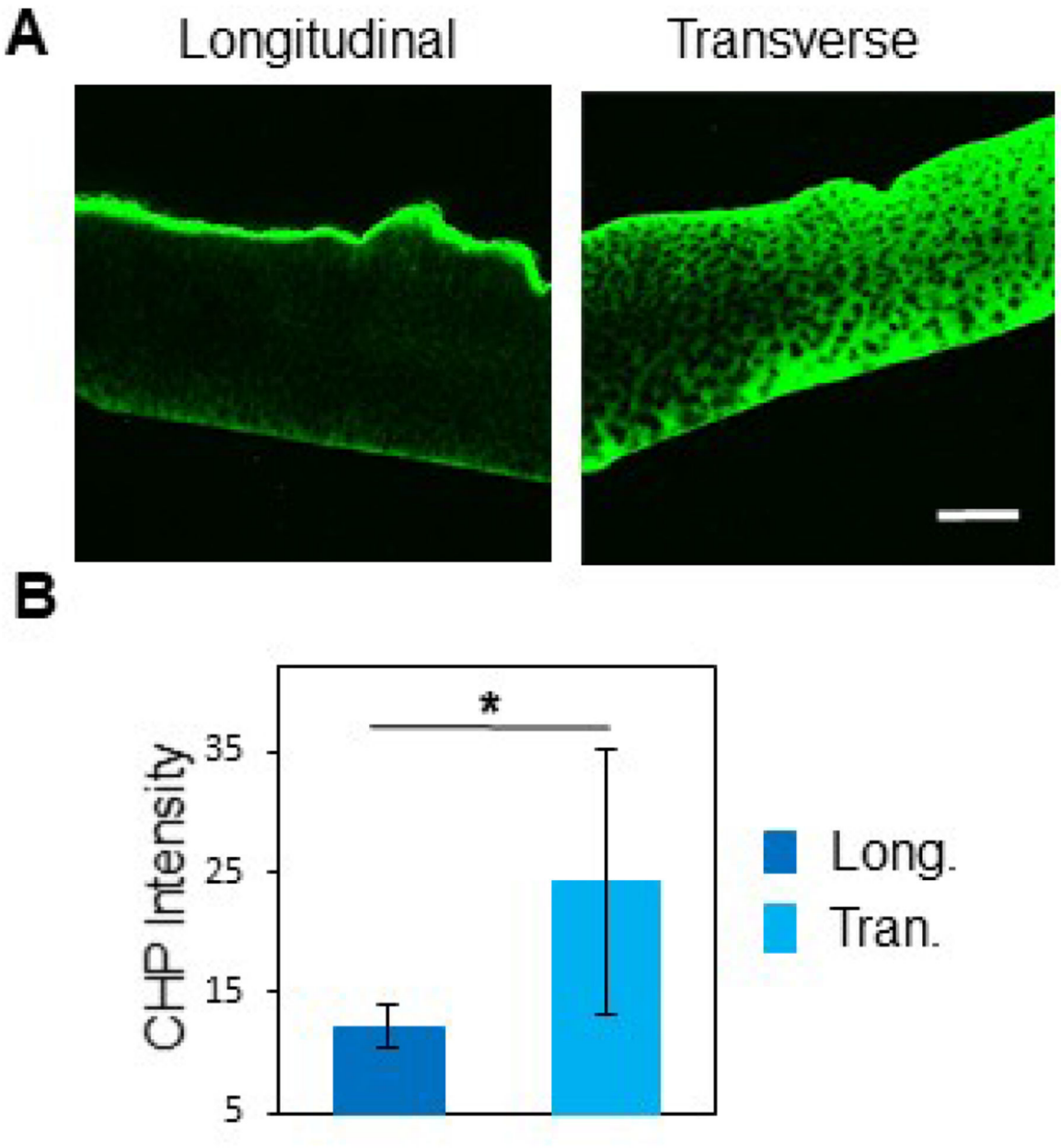

Figure 5. Collagen damage.

Collagen hybridizing peptide (CHP) was used to quantify and visualize collagen damage. A) Specimens worn in the longitudinal and transverse directions were stained with CHP conjugated to fluorescein and imaged with confocal microscopy. Specimens with median fluorescence intensity are shown. Scale bar: 200 μm. B) Quantification of CHP intensity. * indicates $p < 0.05$.

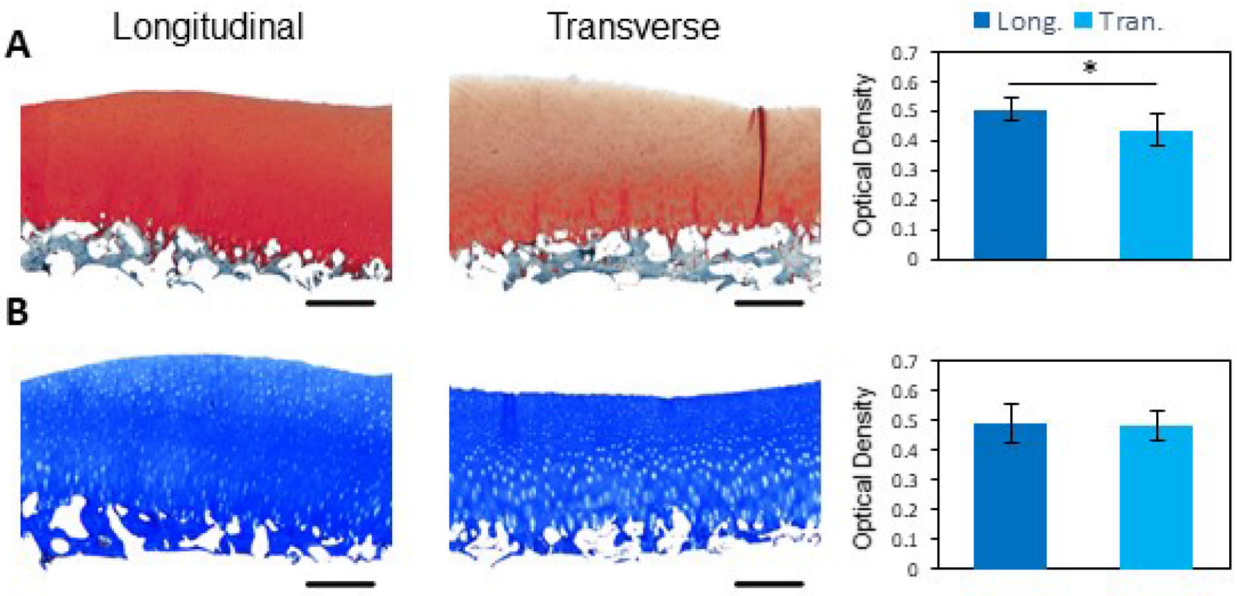

Figure 6. Histological analysis of wear specimens.

A) Safranin-O/Fast Green stain indicating GAG distribution in red and optical density quantification; B) Trichrome stain indicating collagen distribution in blue and optical density quantification. Images represent the median optical density of their respective stains. Scale bar: 500 μm, * indicates $p < 0.05$.