



# The distribution of superficial zone protein (SZP)/lubricin/PRG4 and boundary mode frictional properties of the bovine diarthrodial joint

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## ABSTRACT

The diarthrodial, knee joint is a remarkably efficient bearing system; articulating cartilage surfaces provide nearly frictionless performance with minimal wear. The low friction properties of the cartilage surfaces are due in part to the boundary lubricant, superficial zone protein (SZP); also known as lubricin or proteoglycan 4 (PRG4). In previous work, SZP localization and cartilage friction were examined across the femoral condyles. Studies in the literature have also individually investigated the other tissues that comprise the human knee and four-legged animal stifle joint, such as the meniscus or patella. However, comparisons between individual studies are limited due to the variable testing conditions employed. Friction is a system property that is dependent on the opposing articulating surface, entraining speed, and loading. A cross-comparison of the frictional properties and SZP localization across the knee/stifle joint tissues utilizing a common testing configuration is therefore needed. The objective of this investigation was to determine the friction coefficient and SZP localization of the tissues comprising the three compartments of the bovine stifle joint: patella, patellofemoral groove, femoral condyles, meniscus, tibial plateau, and anterior cruciate ligament. The boundary mode coefficient of friction was greater in tissues of the patellofemoral compartment than the lateral and medial tibiofemoral compartments. SZP immunolocalization followed this trend with reduced depth of staining and intensity in the patella and patellofemoral groove compared to the femoral condyles and tibial plateau. These results illustrate the important role of SZP in reducing friction in the tissues and compartments of the knee/stifle joint.

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## 1. Introduction

Articular cartilage in the diarthrodial joint possesses low friction and wear characteristics due to several mechanisms of lubrication (McNary et al., 2012). While friction is reduced by the formation of fluid films under hydrodynamic and elastohydrodynamic modes of lubrication, low articulation or sliding speeds that occur during the reversal of motion in the swinging leg throughout walking preclude the generation of a fluid film (Neu et al., 2008). Under these conditions, boundary mode lubricants present in the synovial fluid form a molecular monolayer that separate the articulating surfaces and prevent solid-to-solid

contact to reduce friction. Over the years, three main candidates emerged as putative boundary lubricants: superficial zone protein (SZP), hyaluronan, and surface active phospholipids. Studies that employed selective, enzymatic removal of each lubricant demonstrate that SZP is the primary boundary lubricant in articular cartilage (Chan et al., 2010; Jay and Cha, 1999). A preponderance of evidence suggests that hyaluronan synergistically interacts with SZP to enhance lubrication and wear properties, while the physiological role of surface active phospholipids is still under debate (Jay et al., 2007b; Schmidt et al., 2007). SZP (345 kDa) (Schumacher et al., 1994), also known as lubricin (227 kDa) (Swann et al., 1981) and proteoglycan 4 (PRG4, 460 kDa) (Ikegawa et al., 2000), are alternative lubricating isoforms of the *prg4* gene.

SZP is expressed and/or localized within a multitude of tissues distributed throughout the human body. While the majority of SZP in the knee joint is synthesized by the superficial zone articular cartilage and synovium (Schumacher et al., 1999), cells in the

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meniscus (Schumacher et al., 2005), tendon (Rees et al., 2002), ligament (Lee et al., 2008b; Zhang et al., 2011), and infrapatellar fat pad (Lee et al., 2008a) produce SZP as well. SZP has also been demonstrated to be expressed by, and lubricate, the eye lid–corneal interface (Cheriyian et al., 2011; Schmidt et al., 2013), intervertebral disk (Shine et al., 2009), and temporomandibular joint (TMJ) (Koyama et al., 2014; Wei et al., 2010). While the source is unknown, SZP is additionally present in whole blood, plasma, serum, and platelet-rich plasma (Sakata et al., 2015; Su et al., 2001). Patients diagnosed with camptodactyly–arthropathy–coxa vara–pericarditis syndrome (CACV) lack a functional copy of SZP and experience overgrowth of the pericardium, suggestive of SZP's lubricative function in the external lining of the heart (Marcelino et al., 1999). Perhaps most importantly, the loss of SZP in these patients also leads to synovial hyperplasia and precocious joint failure. All these examples illustrate the importance and versatility of SZP as a biological lubricant.

The tribological properties of SZP and various tissues are frequently assayed using a tribometer. The testing parameters are important to the obtained results as friction is a system property (Reeves et al., 2013). No material has an intrinsic friction coefficient as friction is dependent on the counterfacing material, entrainment speed, and normal load among other parameters (Neu et al., 2008). However, under nearly all boundary mode conditions examined, SZP has been shown to reduce friction in cartilage-on-cartilage (Schmidt et al., 2007; Swann et al., 1981), cartilage-on-glass (DuRaine et al., 2009; Gleghorn et al., 2009), and latex-on-glass (Jay and Cha, 1999) interfaces. While many studies in the literature have examined the frictional properties of specific tissues from the diarthrodial joint, to the best of our knowledge no single study has measured and compared the friction characteristics of the different tissues present in the knee. No head-to-head comparison of all knee joint tissues has been reported. As friction measurements between different tribometers can differ due to the

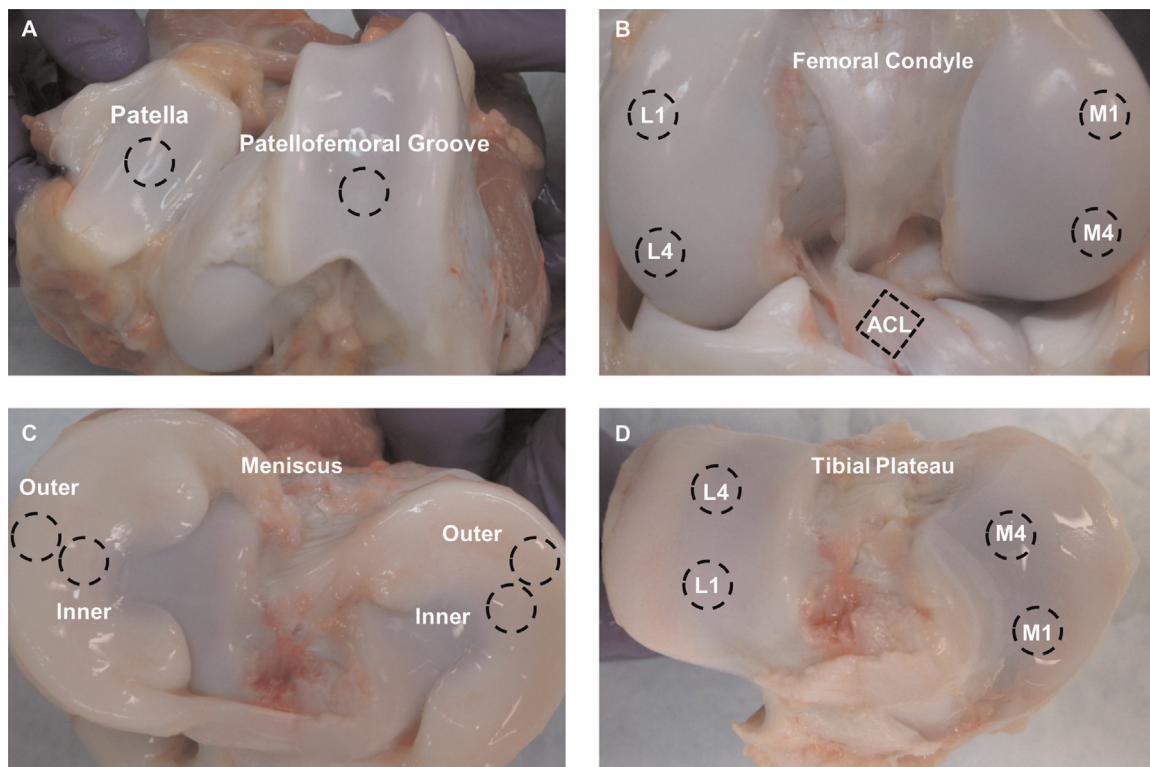
forementioned reasons, a true comparison can only be assessed through a common tribometer operated under identical conditions.

The primary objective of this investigation was to determine the friction coefficients ( $\mu$ ) of the different knee joint tissues using a single, common tribometer. The bovine stifle or knee joint was chosen as a model system since these joints are readily available and contain relatively large amounts of cartilage needed for tissue and cell culture studies. Tissues from the patella, patellofemoral groove, femoral condyle, meniscus, tibial plateau, and the anterior cruciate ligament (ACL) were examined. SZP and glycosaminoglycan distribution throughout the different tissue compartments of the knee joint were assayed by immunohistochemistry and histology, respectively, to identify their contributions towards friction. The results of this study suggest that SZP localization in the different tissues do play a significant role in the friction coefficient of the tissues.

## 2. Materials and methods

### 2.1. Tissue harvest and preparation

Stifle joints from 1 to 3 month-old calves were obtained from an abattoir and dissected under aseptic conditions within 24 h of sacrifice. Full thickness, osteochondral plugs were obtained from the patella, patellofemoral groove, femoral condyle, meniscus, and tibial plateau using a 5 mm diameter coring reamer. Explants were removed from the outer (vascularized) and inner (avascular) rims of the central portion of the lateral and medial menisci as these areas yielded relatively flat samples for tribological testing (Fig. 1C). Anterior and posterior regions of the femoral condyle (Fig. 1B) and tibial plateau (Fig. 1D) were harvested based on previous findings that the friction coefficient varied between these locations along the femoral condyle (Neu et al., 2007). The ACL (Fig. 1B) was dissected using a scalpel. All materials were procured from Life Technologies (Grand Island, NY) unless otherwise noted. Full thickness tissues were stored and washed  $3 \times$  in DMEM/F-12 containing 1% penicillin/streptomycin before incubation in culture medium (DMEM/F-12, 50  $\mu$ g/mL ascorbate 2-phosphate (Sigma, St. Louis, MO), 0.1%



**Fig. 1.** Harvest locations of the tissues from the bovine stifle joint. Explant tissues were obtained from the patella and patellofemoral groove (A); medial anterior (M1), medial posterior (M4), lateral anterior (L1), and lateral posterior (L4) regions of the femoral condyles (B); ACL (B), inner and outer regions of the central, medial and lateral menisci (C); and M1, M4, L1, and L4 regions of the tibial plateau (D).



bovine serum albumin (BSA, Sigma), 1% penicillin/streptomycin, 0.5% Fungizone, and 1% ITS+ Premix (BD Biosciences, Bedford, MA)) for 2–3 days. Friction tests were performed on days 2 and 3.

Prior to each friction test, the full thickness 5 mm diameter explants were trimmed to a thickness of ~1 mm in a custom, cutting jig before testing the surface zone of articular cartilage (Neu et al., 2007). The femoral side of the meniscus was tested. The ACL was cut into approximately 4.43 mm × 4.43 mm square pieces, with a thickness of ~1 mm, to match the surface area of 5 mm diameter explants. An additional set of cartilage explants ( $n=10$ ) were obtained from the anterior and posterior regions of the medial condyle. To examine the effects of cartilage zone on the frictional properties, three consecutive 400  $\mu$ m cartilage sections were cut (surface, superficial zone, and middle zone layer) and tested on the side closest to the surface zone.

## 2.2. Friction testing

The friction coefficient was determined using a pin-on-disk tribometer operated in the boundary lubrication regime in reciprocating sliding mode as described previously (Neu et al., 2007). Briefly, cartilage samples were affixed to acrylic pins using ethyl cyanoacrylate glue and brought into contact with a polished glass disk while fully immersed in phosphate buffered saline (PBS). Prior to the initiation of each friction test, the sample was allowed to equilibrate under the applied load (0.1 MPa) in an unconstrained test configuration for 2 min to minimize any fluid effects during testing. The test duration of each friction experiment was fixed at 5 min at a sliding speed of 0.5 mm/s. Data was collected at a rate of 10 Hz (DAQ-View, IOTech, Cleveland, OH) and processed using a standard software package (Microsoft, Seattle, WA). These experimental conditions have been previously used to investigate the effects of SZP expression levels on the coefficient of friction of femoral condyle articular cartilage explants (DuRaine et al., 2009).

## 2.3. Histology and immunohistochemistry (IHC)

Freshly isolated cartilage from the patellofemoral groove, patella, medial meniscus, medial anterior femoral condyle, medial anterior tibial plateau, and the ACL were fixed in Bouin's solution (Sigma) for 24 h. The samples were then embedded in paraffin and sectioned at 4  $\mu$ m intervals. Paraffin sections were deparaffinized using xylene and rehydrated with graded ethanol, quenched of peroxidase activity with hydrogen peroxide, and blocked with 1% BSA. Sections were either stained with toluidine blue or probed for SZP using monoclonal antibody (mAb) S6.79 (a generous gift from Dr. T. Schmid, Rush Medical College, Chicago, IL) (Su et al., 2001). Samples were treated overnight at 4 °C with a 1:1000 dilution of SZP mAb, followed by incubations with biotinylated anti-mouse immunoglobulin G (IgG) diluted 1:3000 in 1% BSA (Vector Laboratories, Burlingame, CA) and VECTASTAIN ABC reagent (Vector Laboratories) for 30 min each. Visualization was achieved through diaminobenzidine treatment (ImmPACT DAB, Vector Laboratories) for 20 s prior to rinsing. Cover slips were mounted onto each slide using Eukitt mounting medium (Sigma).

## 2.4. Statistics

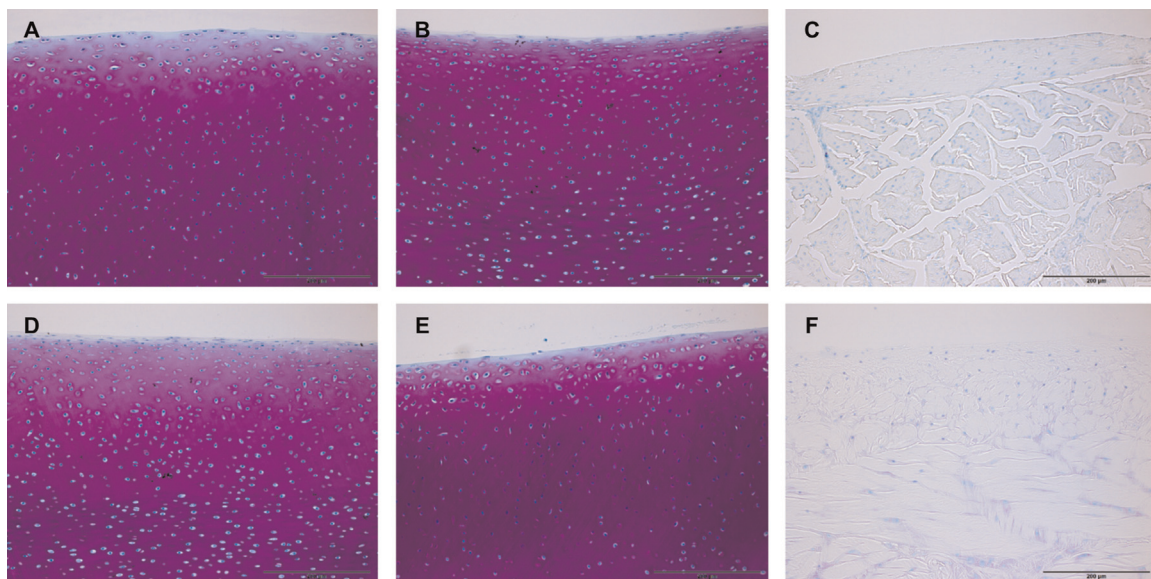
For all friction assays,  $n=10$ –11 samples were examined. Representative samples of histology and IHC results are shown from  $n=4$  samples. All values were reported as mean  $\pm$  standard deviation (SD). Single, pairwise significance comparisons were assessed using a two-tailed, Student's *t*-test (Microsoft Excel). Multiple comparisons were evaluated through analysis of variance (ANOVA) followed by Tukey's *post hoc* test (JMP10). *p*-Values less than 0.05 were considered significant. For an ANOVA where no significant difference between the groups was determined, the *p*-Value for this test was shown. If an ANOVA test revealed a significant difference between groups, the *p*-Values generated during the *post hoc* test were reported. In all data charts, groups not connected by the same letter were determined to be significantly different.

## 3. Results

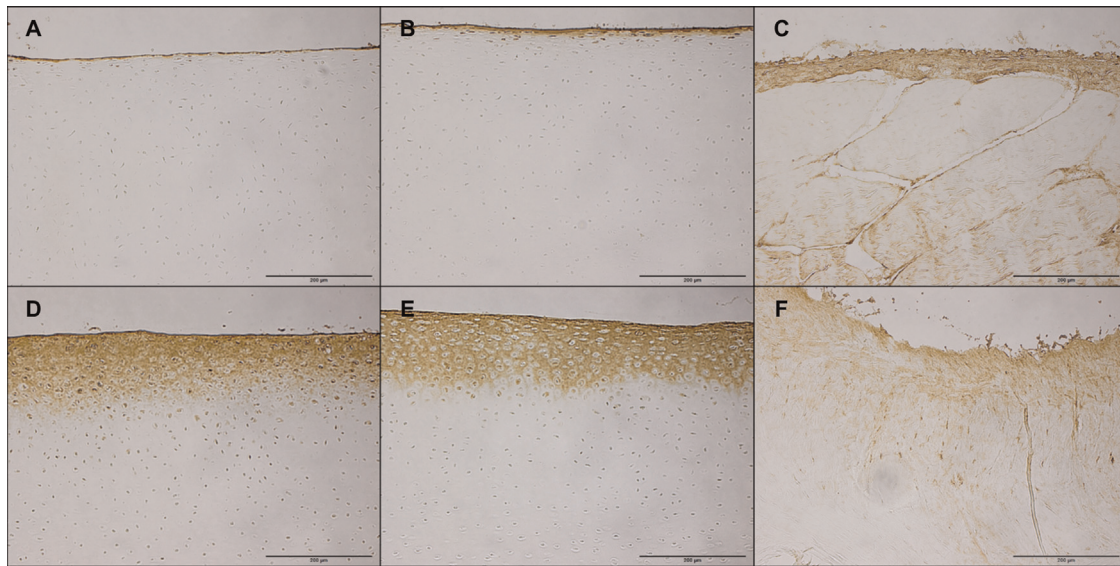
### 3.1. Histology and SZP IHC

Intense metachromatic, toluidine blue staining demonstrated the rich, sulfated glycosaminoglycan content of the articular cartilages obtained from the patella (Fig. 2A), patellofemoral groove (Fig. 2B), medial anterior femoral condyle (Fig. 2D), and medial anterior tibial plateau (Fig. 2E). While no apparent differences in glycosaminoglycan content were observed between the patella, patellofemoral groove, and tibial plateau cartilages, the femoral condyle cartilage displayed slightly less metachromasia. All articular cartilage explants (Fig. 2A, B, D, and E) exhibited a thin region of decreased metachromatic intensity at the surface, whereas glycosaminoglycans were not detected in the ACL (Fig. 2C). Faint staining was observed in the meniscus (Fig. 2F) as expected due to the low levels (~2.5% dry weight) of sulfated glycosaminoglycans present (Herwig et al., 1984; Proctor et al., 1989).

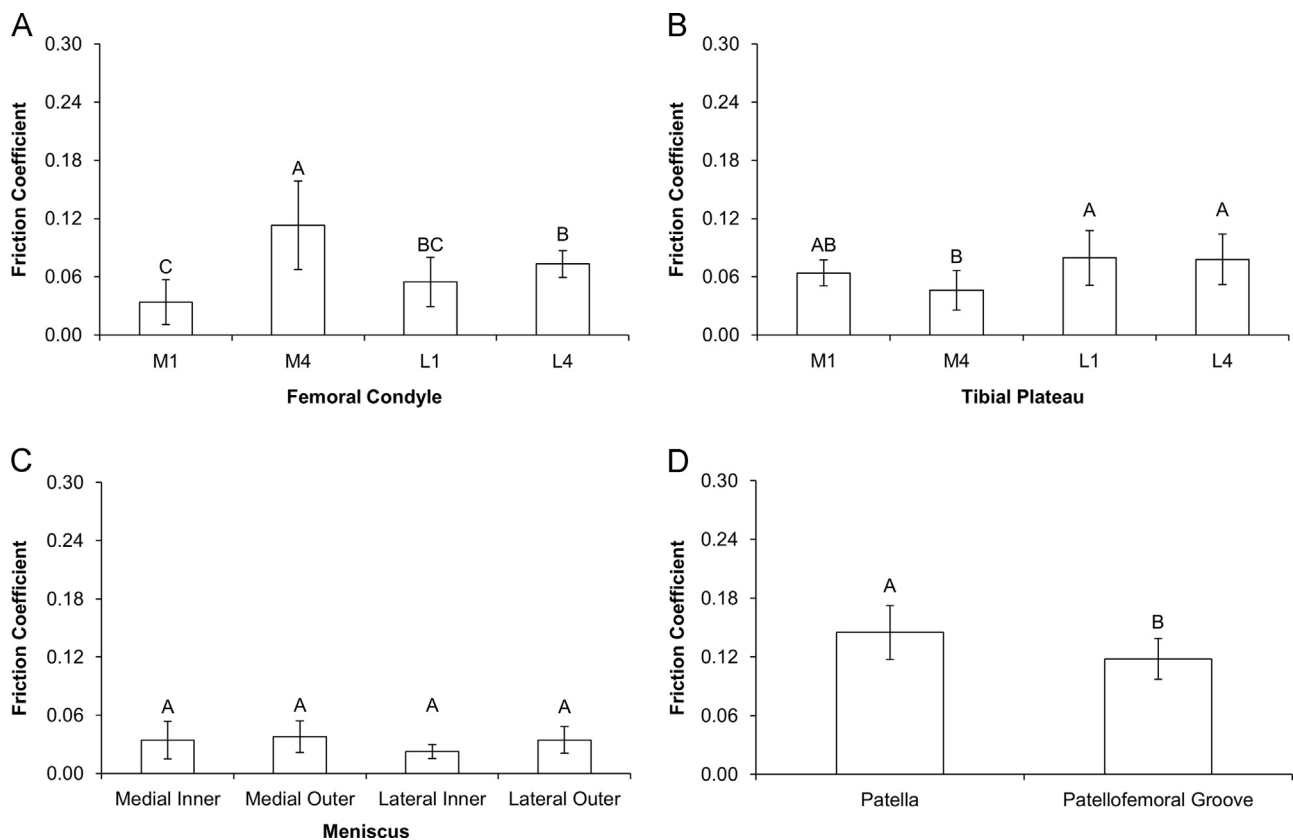
All knee joint tissues stained positive for SZP, with staining localized primarily at the articular surface (Fig. 3). The depth and intensity of SZP immunolocalization was greatest in the femoral condyle (Fig. 3D) and tibial plateau cartilages (Fig. 3E). In contrast, the depth of SZP staining was limited to the articular surfaces of the patellar (Fig. 3A) and patellofemoral groove (Fig. 3B) cartilages. Compared to the articular cartilage samples, SZP staining was more diffuse in the ACL (Fig. 3C) and meniscus (Fig. 3F) tissues examined. In the ACL, SZP staining was observed at the collagen fiber bundle interfaces.



**Fig. 2.** Toluidine blue staining of the tissues that comprise the bovine stifle joint. Paraffin-embedded sections of the patella (A), patellofemoral groove (B), ACL (C), medial anterior (M1) femoral condyle (D), medial anterior (M1) tibial plateau (E), and central, inner meniscus (F) were stained with toluidine blue to detect sulfated glycosaminoglycans (Scale bar: 200  $\mu$ m).



**Fig. 3.** SZP immunostaining of the tissues that comprise the bovine stifle joint. Paraffin-embedded sections of the patella (A), patellofemoral groove (B), ACL (C), medial anterior (M1) tibial plateau (D), medial anterior (M1) tibial plateau (E), and central, inner meniscus (F) were stained with monoclonal antibody S6.79 to detect SZP localization (Scale bar: 200  $\mu$ m).



**Fig. 4.** Coefficients of friction of articulating cartilage surfaces of the bovine stifle joint. The boundary mode friction coefficient was measured across the femoral condyles (A), tibial plateau (B), and meniscus (C). The coefficient of friction was also measured at the central regions of the patella and patellofemoral groove (D). Medial anterior (M1), medial posterior (M4), lateral anterior (L1), and lateral posterior (L4) regions of the femoral condyle and tibial plateau were tested. The inner and outer regions of the medial and lateral menisci were also assayed. Values are presented as mean  $\pm$  SD. Multiple group comparisons (AC) were evaluated for statistical significance by ANOVA with a Tukey's *post hoc* test. Groups not connected by the same letter were determined to be significantly different ( $p < 0.05$ ). A Student's *t*-test was performed for pairwise comparison (D).

### 3.2. Friction characteristics

The coefficients of friction of cartilage explants obtained from the medial anterior (M1), lateral anterior (L1), and lateral posterior (L4) regions of the femoral condyle were significantly lower than

the medial posterior (M4) region ( $p < 0.016$ ) (Fig. 4A). In contrast, the friction coefficient of the medial posterior (M4) region of the tibial plateau was less than both lateral regions (L1 and L4) ( $p < 0.05$ ) (Fig. 4B). The medial anterior (M1) tibial cartilage friction coefficient was not significantly different from the other

**Table 1**

The boundary mode friction coefficients of the articulating tissues that comprise the tibiofemoral and patellofemoral compartments of the bovine stifle joint. The tissue friction coefficients of each compartment were averaged to determine the compartmental friction coefficient. The data (mean  $\pm$  SD) shown here in tabular forms are presented graphically in Figs. 4 and 5A. Please refer to these figures for statistical comparisons.

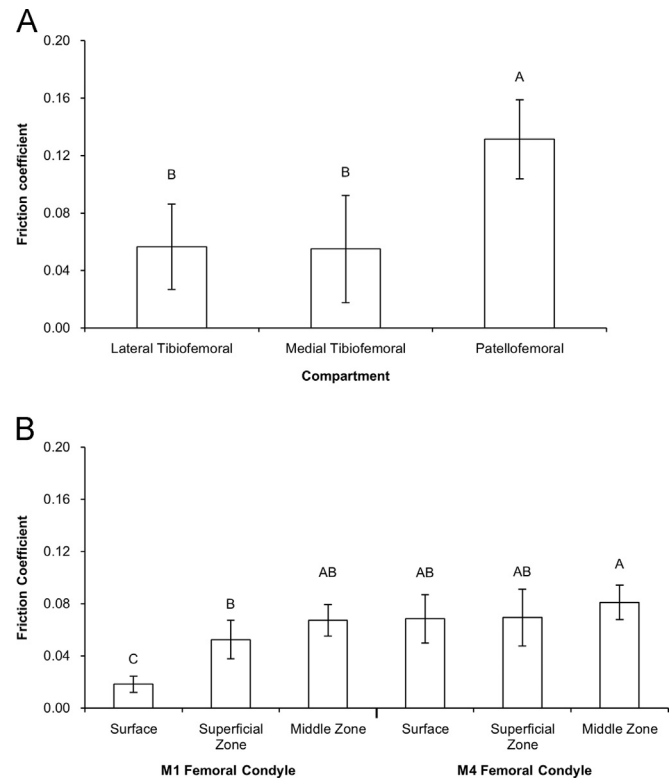
Compartment	Tissue	Location	Friction coefficient	
			Tissue	Compartment
Medial tibiofemoral	Femoral condyle	M1	0.034 $\pm$ 0.023	0.057 $\pm$ 0.030
		M4	0.113 $\pm$ 0.046	
	Meniscus	Inner	0.034 $\pm$ 0.019	
		Outer	0.038 $\pm$ 0.016	
	Tibial plateau	M1	0.064 $\pm$ 0.013	
		M4	0.046 $\pm$ 0.020	
Lateral tibiofemoral	Femoral condyle	L1	0.055 $\pm$ 0.025	0.055 $\pm$ 0.037
		L4	0.073 $\pm$ 0.014	
	Meniscus	Inner	0.023 $\pm$ 0.007	
		Outer	0.034 $\pm$ 0.014	
	Tibial plateau	L1	0.079 $\pm$ 0.028	
		L4	0.078 $\pm$ 0.026	
Patellofemoral	Patella	Central	0.145 $\pm$ 0.027	0.131 $\pm$ 0.027
	Patellofemoral groove	Central	0.118 $\pm$ 0.021	

regions tested ( $p > 0.30$ ). No significant differences in frictional properties were observed between the lateral and medial menisci, or the inner and outer sections as well ( $p = 0.0979$ ) (Fig. 4C). The boundary mode friction coefficient of the patellofemoral groove cartilage was significantly lower than the patellar cartilage ( $p < 0.009$ ) (Fig. 4D). Lastly, the coefficient of friction for the ACL was determined to be  $0.17 \pm 0.07$ . The friction coefficient values displayed in Fig. 4 are listed in Table 1.

When the tissues were grouped by joint compartment (tibiofemoral tissues: femoral cartilage, meniscus, and tibial cartilage; patellofemoral tissues: patella, patellofemoral groove) and compared, the frictional coefficient of the lateral and medial tibiofemoral compartments were significantly lower than the patellofemoral compartment ( $p < 0.0001$ ) (Fig. 5A). On average, there was no difference between the lateral and medial compartments ( $p = 0.9626$ ). The frictional properties of the surface and middle zone were also evaluated as the chondrocytes from these regions are more frequently being employed in tissue engineering applications (Peng et al., 2014). The friction coefficient of the surface zone, articular cartilage from the M1 region of the femoral condyle was significantly lower than the surface, superficial zone (400  $\mu$ m depth), and middle zone cartilages (800  $\mu$ m depth) of the M4 region ( $p < 0.0001$ ) (Fig. 5B).

#### 4. Discussion

The objective of this investigation was to characterize and compare the friction coefficient and SZP localization of the tissues that comprise the articulating surfaces of the knee joint: patella, patellofemoral groove, femoral condyle, meniscus, and tibial plateau. The bovine stifle joint was examined as an analogous surrogate since it is readily available and provides relatively large quantities of tissue for study. While the frictional properties and SZP immunolocalization in each of the aforementioned tissues that comprise the knee/stifle joint have been individually studied, a complete survey is needed for accurate comparisons. The friction values of the knee/stifle joint tissues in the literature were



**Fig. 5.** The aggregate friction coefficients of the patellofemoral and tibiofemoral compartments of the bovine stifle joint, and depth dependent frictional properties of femoral articular cartilage. The mean friction coefficient of the tissues that comprise each joint compartment was computed (A). Friction coefficients were also measured at different depths of cartilage obtained from the medial anterior (M1) and medial posterior (M4) regions of the femoral condyle (B). Three consecutive sections (400  $\mu$ m thick) from the surface of the femoral condyles were removed and tested. These slices were labeled surface (0  $\mu$ m depth), superficial zone (400  $\mu$ m depth), and middle zone (800  $\mu$ m depth). Values are presented as mean  $\pm$  SD. All multiple statistical comparisons were assessed by a one-way ANOVA and Tukey's *post hoc* test. Groups not connected by the same letter were significantly different ( $p < 0.05$ ).

measured under variable testing conditions. A material does not possess an intrinsic friction coefficient as friction is a system property that depends on the opposing articulating surface, entraining or sliding speed, loading, and lubricant composition. This investigation sought to fill this gap and provide a survey of the friction coefficients of the articulating tissues of the knee/stifle joint. The tissues of the patellofemoral compartment had higher boundary mode friction coefficients than the tissues of the lateral and medial tibiofemoral compartments. SZP immunolocalization also followed this trend, with greater depth and intensity of staining in the tibiofemoral compartment tissues. These results bolster the evidence of the role SZP plays in reducing friction in knee/stifle joint tissues.

Topographical differences in friction were observed across the tibial plateau, where the friction coefficient at the M4 region was significantly lower than the friction values obtained from the lateral half (L1 and L4) of the tibial plateau, and trended lower than the medial anterior region (M1) (Fig. 4B). Interestingly, this pattern was reversed in the femoral condyles, where the friction coefficient of the medial anterior region (M1) was significantly lower than the medial posterior (M4) and both lateral regions (L1 and L4) (Fig. 4A). However, it is important to note that the differences in the friction coefficient between the anterior and posterior regions are much greater across the medial femoral condyle (> 200%) than the medial tibial plateau (38%). This pattern of frictional properties in the medial tibiofemoral compartment



follows the observed trends in contact area distribution. Whereas the contact area is primarily concentrated over the anterior half of the medial femoral condyle (Neu et al., 2007), loading is distributed over a greater area of the medial tibial plateau (Fukubayashi and Kurosawa, 1980; Taylor et al., 2011). In addition, no differences in frictional properties were observed between the inner (avascular) and outer (vascularized) rims of the medial and lateral menisci (Fig. 4C). Comparisons to the anterior and posterior regions of the meniscus were unable to be made as the size and curvature of these regions prevented the obtaining of planar, 5 mm diameter explants for testing. Lastly, the friction coefficient of cartilage at the M1 location of the femoral condyles significantly increased from the surface to the superficial zone, and superficial zone to middle zone (Fig. 5B). Interestingly, there were no significant differences between the cartilage zones of explants from the M4 location. Collectively, these observations highlight the importance of topographical location when studying the frictional properties of connective tissues.

Tissue mechanics are an additional factor that influences tribological properties. Moore and Burris (2015) examined the influence of tissue material properties on lubrication by comparing the femoral condyles and tibial plateau of bovine stifle joints. After examining multiple material parameters, they found that fluid film-mediated friction improved (i.e. decreased) with greater equilibrium contact modulus, greater tensile modulus, and reduced permeability. While the bulk mechanical properties modulate friction predominantly through fluid film effects, such as hydrodynamic lubrication and interstitial fluid depressurization, its effects on boundary mode friction are unknown. Confounding this issue further is the observed disparity between the compressive properties of the bulk and surface of articular cartilage. Whereas the aggregate modulus of the bulk tissue varies between 0.47 and 0.90 MPa (Athanasios et al., 1991), the compressive modulus of the surface is an order of magnitude lower at 46–79 kPa (Park et al., 2004; Schinagl et al., 1997). The friction forces that arise from solid-to-solid phase contact between opposing surface asperities in the boundary mode, are the cumulative effects of adhesion, asperity deformation, and plowing from asperities and wear particles (Chan et al., 2010; Suh and Sin, 1981). While the superficial tissue stiffness will likely modulate asperity deformation and plowing, surface roughness and adhesive forces need to be considered as well. In order to sort through these various tribological mechanisms, future studies need to consider the surface material properties of the interfacing tissues as well (Moore and Burris, 2015).

This investigation observed that the medial anterior (M1) regions of the femoral condyle, tibial plateau, and central regions of the meniscus all displayed low coefficients of friction,  $\sim 0.04$ , and detected higher friction values in the patella, patellofemoral groove, and ACL (Fig. 4). When these results were compared to values published in the literature, there was mixed agreement. Baro et al. (2012) observed a similar coefficient of friction of  $\sim 0.04$  in the medial meniscus. Comparable friction coefficients for the femoral condyles have been published: Basalo et al. (2007) reported a  $\mu_{120\text{ s}}$  of 0.035, Caligaris and Ateshian (2008) determined an initial  $\mu_{\text{eff}}$  of 0.01, and a kinetic friction coefficient of  $\sim 0.08$  was described by Waller et al. (2013). In contrast to the coefficient of 0.12 reported here for patellofemoral groove cartilage, others have measured values ranging between 0.2 and 0.28 (Gleghorn et al., 2009; Schmidt et al., 2007). A greater disparity emerged for the patellar cartilage, where Kumar et al. (2001) related a friction coefficient of 0.0028. Overall, the published friction coefficients of patellofemoral groove cartilage (Gleghorn et al., 2009; Schmidt et al., 2007) are consistently higher than the femoral condyles (Forster and Fisher, 1996; Neu et al., 2007; Waller et al., 2013). Despite the various differences in testing configurations cited, the literature corroborates the overall trends

identified in this investigation. An assortment of tribometer testing parameters has been employed to measure cartilage friction. Tribometers have differed by configuration (pin-on-disk, disk-on-disk, and annulus-on-disk), loading type (stress- or strain-controlled compression), counterface material (glass or cartilage), loading magnitude, articulation/sliding speed, lubricant (saline, synovial fluid, hyaluronan, SZP), and sliding duration. The multiplicity of tribological testing parameters utilized in the literature would complicate the performance and interpretation of a comprehensive meta-analysis. In addition, a paucity of reported friction properties for tissues such as patellar cartilage would reduce statistical power and possibly preclude any meaningful conclusions from being drawn. Direct, head-to-head assessment remains the experimental gold standard, and thusly motivated this investigation to evaluate the friction properties of knee/stifle joint tissues on a common tribometer.

As the main focus of this investigation was to examine the primary articulating tissues of the knee/stifle joint, the ACL was included to provide a limited comparison to sinew tissues. The ACL was the only tissue examined that possessed both strong staining for SZP (Fig. 2C) and a high coefficient of friction (0.17). Although ligaments and tendons are loaded in tension *in vivo*, compressive forces develop in regions where the sinew slides over objects like a pulley, such as bone (Theobald et al., 2012). While this result is most likely not a true reflection of the frictional properties of the ACL, as the tissue was not stretched in tension during friction testing, it provides a rudimentary preview of the frictional forces the ACL would experience under pulley-generated, compressive loading. The friction coefficient reported here for ACL is approximately five-fold greater than the frictional properties of bovine, deep flexor tendon ( $\sim 0.005$ – $0.035$ ) described by Theobald et al. (2012). However, a difference of this magnitude is likely, given that the tendon was tested under synovial fluid lubrication (a rich source of SZP) as opposed to saline. Given the resemblance in SZP immunolocalization at the tissue surface and collagen bundle interfaces, there are likely to be similarities in the frictional properties between tendon and ligaments as well (Lee et al., 2008b; Rees et al., 2002; Sun et al., 2006).

SZP is a critical boundary lubricant for articular cartilage; opposing premature degeneration and wear (Jay et al., 2007a; Marcelino et al., 1999). As measured in the aggregate, the friction coefficient of the patellofemoral compartment was greater than the medial and lateral tibiofemoral compartments. Conversely, the intensity and depth of SZP immunolocalization in the patellofemoral compartment was less than the tibiofemoral compartment. The results of this investigation suggest a common level of SZP synthesis and boundary mode friction among tissues of the same knee compartment. Although there are local, topographical differences across tissues due to contact forces, it stands to reason that articulating tissues share macroscopic, tribological properties as a mechanism to reduce friction and wear.

## Conflict of interest statement

The authors confirm that there are no conflicts of interest.

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