Increased Friction Coefficient and Superficial Zone Protein Expression in Patients With Advanced Osteoarthritis

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Objective. To quantify the concentration of superficial zone protein (SZP) in the articular cartilage and synovial fluid of patients with advanced osteoarthritis (OA) and to further correlate the SZP content with the friction coefficient, OA severity, and levels of proinflammatory cytokines.

Methods. Samples of articular cartilage and synovial fluid were obtained from patients undergoing elective total knee replacement surgery. Additional normal samples were obtained from donated body program and tissue bank sources. Regional SZP expression in cartilage obtained from the femoral condyles was quantified by enzyme-linked immunosorbent assay (ELISA) and visualized by immunohistochemistry. Friction coefficient measurements of cartilage plugs slid in the boundary lubrication system were obtained. OA severity was graded using histochemical analyses. The concentrations of SZP and proinflammatory cytokines in synovial fluid were determined by ELISA.

Results. A pattern of SZP localization in knee cartilage was identified, with load-bearing regions exhibiting high SZP expression. SZP expression patterns were correlated with friction coefficient and OA severity; however, SZP expression was observed in all samples at the articular surface, regardless of OA severity. SZP expression and aspirate volume of synovial fluid were higher in OA patients than in normal controls. Expression of cytokines was elevated in the synovial fluid of some patients.

Conclusion. Our findings indicate a mechanochemical coupling in which physical forces regulate OA severity and joint lubrication. The findings of this study also suggest that SZP may be ineffective in reducing joint friction in the boundary lubrication mode at an advanced stage of OA, where other mechanisms may dominate the observed tribological behavior.

Superficial zone protein (SZP), also known as lubricin or proteoglycan 4 (PRG4), is a mucinous glycoprotein secreted by tissues that line the interior surfaces of animal joints (1). Down-regulation of SZP has been associated with the pathogenesis of osteoarthritis (OA) (2). SZP may act as a chondroprotective barrier against direct solid-to-solid contact in joints where the kinematic conditions are conducive to surface sliding in the boundary lubrication mode, characterized by the formation of an adsorbed molecular layer conformal with the articular tissue surface topography (3). In the absence of a strongly adsorbing, continuous, self-replenishing boundary lubricant layer, intermittent asperity–asperity interactions lead to rapid deterioration of the joint surface by various mechanical wear processes, such as adhesion, abrasion, surface fatigue, and delamination.

A striking pattern of SZP localization in bovine cartilage, with load-bearing regions exhibiting increased SZP expression, was identified in a previous study (3). Regional SZP patterns were regulated by a mechanical shear force through transforming growth factor β receptor type I (TGFβRI) kinase activity and subsequent phospho-Smad2/3 activity, and were correlated with tribological behavior. Direct relationships were observed between the highest SZP expression, the maximum
contact pressure, and the lowest friction coefficient. However, it is unknown whether regional SZP expression patterns also exist in human joints.

OA is a degenerative joint disease and the most common form of arthritis, affecting tens of millions of people in the US (4). The etiologies of this disease are largely unknown, but likely involve multiple factors, including a biochemical imbalance between catabolic and anabolic factors and a progressive surface degradation caused by mechanical wear (3). In the most extreme cases, the cartilage may be completely worn away, resulting in direct bone-to-bone contact that requires late-stage treatment strategies, including total knee arthroplasty. Although the decrease in synovial fluid lubricin concentration observed after anterior cruciate ligament injury may increase the risk of wear-induced joint damage due to the lack of effective boundary lubrication (5), whether SZP is present in cartilage and synovial fluid at advanced stages of OA is unknown. Thus, the objective of this study was to investigate the localization and concentration of SZP in the articular cartilage and synovial fluid of patients with advanced OA and to correlate the SZP content with the articular surface friction coefficient, OA severity, and levels of proinflammatory cytokines.

MATERIALS AND METHODS

Tissue acquisition. Multiple human tissue sources were used to study joint biochemistry and tribology. Twenty-one patients (11 men and 10 women) with an average age of 66 years (range 44–79 years) who were undergoing elective surgery for joint replacement were included in the study and served as the primary tissue source. The study was approved by the Institutional Review Board of the University of California, Davis. Both osteochondral tissue and synovial fluid were collected at the time of surgery. Cylindrical osteochondral samples (with a diameter of 5 mm and a length of 5 mm) were harvested from standardized locations on the femur (lateral anterior, lateral posterior, medial anterior, and medial posterior) using a coring reamer and custom cutting jig (Figure 1A) (3). Lateral anterior and medial anterior locations were from joint regions of relatively high contact pressure, whereas lateral posterior and medial posterior locations were from joint regions of relatively low contact pressure. Major advantages with this approach are reduced (e.g., genetic) variation among multiple OA tissue donors and the possibility of sampling from a continuum of structural damage (minimal to advanced) within a single subject’s joint. Thus, 3 samples from each location were used for protein quantification, histochemical analysis, and tribological testing (as described below). Samples were numerically labeled and stored at −80°C until used for further study.

Additional samples were obtained from donated body program and tissue bank sources for a limited comparison with representative normal tissue. Tissue was harvested as described above from subjects obtained through the donated body program following radiographic screening for signs of cartilage degeneration. Two subjects (both women) with an average age of 54 years (range 52–56 years) were included. Additionally, allograft material was obtained from a single subject. Osteochondral samples (with a diameter of 8 mm and a thickness of 5 mm) from this subject were harvested from the lateral anterior and lateral posterior locations and used for histochemical analysis.

SZP localization. Protein was extracted from tissue samples using a tissue homogenizer and 1 ml of 4M guanidine HCl (pH 7.2) supplemented with 0.2% CHAPS, 10 mM EDTA, 0.05M Tris, and protease inhibitor cocktail (Sigma-Aldrich). Homogenized tissue was centrifuged at 14,000g for 10 minutes. The supernatant was buffer exchanged with 6M urea containing 0.05M Tris (pH 7.4) using Centricon filter devices (Millipore).

Enzyme-linked immunosorbent assay (ELISA) was used to analyze the SZP content (µg/ml) in samples (compared with bovine SZP purified from explant culture systems and synovial fluid). Briefly, samples were analyzed by a sandwich ELISA using peanut lectin as a capture reagent and an anti-SZP monoclonal antibody (mAb) (6). Black 96-well plates were coated overnight at 4°C with 50 µl of 1 µg/ml peanut lectin (E-Y Laboratories) in 50 mM sodium carbonate buffer (pH 9.5) and then blocked for 1 hour at room temperature with bovine serum albumin (BSA) in the same buffer. Two-fold serial dilutions of culture medium in phosphate buffered saline (PBS), 0.05% Tween, and 10 mM EDTA (PBSTE) were incubated with the lectin-coated plate for 1 hour. The plate was washed with PBSTE and incubated overnight with 2 µg/ml of S6.79 mAb to SZP (6). After washing with PBSTE, a secondary anti-mouse IgG horseradish peroxidase (HRP) conjugate (no. PI31432; Thermo) was incubated with the plate at a 1:3,000 dilution for 1 hour at room temperature. The plate was washed, and a chemiluminescent HRP SuperSignal ELISA Femto substrate (no. PI37075; Thermo) was added for 1 minute. Chemiluminescence was measured in a multilabel plate reader. Concentration measurements were based on serial dilutions of purified SZP (1) using a bicinechonic acid protein assay (no. PI23235; Thermo) with BSA as a protein standard. Importantly, confirmatory spiking experiments (through the addition of known amounts of purified SZP to samples) for this ELISA yielded recoveries of 109.0 ± 6.2% and 88.6 ± 10.6% for synovial fluid and cartilage extracts, respectively, and were considered sufficient for the experiments described herein. Finally, SZP concentrations were obtained in units of µg/cm² using the final supernatant volume and the articular surface area of the sample, and computed as the mean ± SEM of all joints harvested from each location.

For immunohistochemistry, tissue sections from joints were fixed in Bouin’s solution for 24 hours, followed by paraffin embedding and sectioning. Immunostaining was performed according to a standard method using S6.79 (1:5,000) as the primary antibody (6) and an ABC kit (Vector Laboratories) with mouse IgG secondary antibody for signal detection.

Friction experiments. To examine whether the SZP expression pattern was related to mechanical effects, friction tests were performed with plugs harvested from different
cartilage locations. All of the friction tests were carried out under boundary lubrication sliding conditions using a pin-on-disk tribometer operated in reciprocating mode. Superficial sections of thickness equal to 4 mm were removed from plugs (harvested as described above), immediately affixed to acrylic pins using ethyl cyanoacrylate, and then used in the friction experiments. The articular surface was brought into contact with a polished glass disk while fully immersed in PBS. The average contact pressure of 0.1 MPa that was applied in all of the tests is within the physiological range that occurs during

Figure 1. Correlation of superficial zone protein (SZP) expression and friction coefficient with histologic scores and femoral condyle location in samples from patients with osteoarthritis (OA). A, Standardized lateral anterior (LA), medial anterior (MA), lateral posterior (LP), and medial posterior (MP) locations of condyles from which samples were harvested. B, Surface expression of SZP (quantified relative to bovine standards) and friction coefficient in samples from patients with OA. SZP expression and friction coefficient varied significantly with OA score. Medial anterior locations exhibited much higher SZP expression and friction coefficients compared with medial posterior, lateral posterior, and lateral anterior locations (P < 0.011 for SZP expression; P < 0.015 for friction coefficient). Values are the mean ± SEM (n = 21 patients). C, Surface expression of SZP (quantified relative to bovine standards) and friction coefficient in samples from normal subjects. The strong correlation of SZP concentration and friction coefficient with OA score observed in the samples from patients with OA was not observed in the samples from normal subjects. Values are the mean ± SEM (n = 2 subjects).
walking (7). Prior to the initiation of sliding, the sample was allowed to equilibrate for 2 minutes under the applied normal load to minimize fluid effects during testing. Thus, sliding occurred in the boundary lubrication system using a standard set of load and speed conditions that were applied to all samples tested and enabled relative differences to be determined (3,8,9). Data processing was accomplished with a standard software package (Microsoft Excel). Data from the first 60 seconds of sliding were used to compute a mean ± SEM friction coefficient for each femoral condyle location.

**OA severity.** OA severity was assessed based on histochemical analysis and a standard OA cartilage damage scoring system (10), which includes 6 grades indicating the depth of the lesion and 4 stages reflecting the extent of OA over the joint surface. Tissue sections from fixed samples described previously were stained with hematoxylin and eosin (H&E) and toluidine blue. The OA score (grade × stage) was assessed by 2 independent observers and averaged for correlation to immunolocalization and tribological data.

**Analysis of synovial fluid.** To investigate biochemical relationships between SZP expression and OA severity, synovial fluid was further analyzed for the concentration of OA-related cytokines. The SZP content in synovial fluid was serially diluted and analyzed by ELISA, as described above. The total volume of synovial fluid aspirated from the joint was determined, and the presence of blood in samples was visually detected. To investigate potential degradation resulting from OA as detected by mAb S6.79, SZP was visualized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis with subsequent immunoblotting, following standard procedures (3). Importantly, the same amount of SZP was loaded in each lane (based on SZP ELISA results), with OA patients having higher SZP content, and therefore requiring fewer relative loads per lane, compared with normal subjects. Concentrations of tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) were determined using Quantikine kits (R&D Systems). A quantitative sandwich enzyme immunoassay was used with mAb specific for TNFα and IL-1β in addition to cytokine standards. Because the OA score varied within the joint, the maximum OA score for each joint was used as an independent variable.

**Statistical analysis.** Differences in OA score, SZP expression, and friction coefficient at different femoral condyle locations were determined using a one-way analysis of variance with Fisher’s protected least significant difference post hoc test. P values less than 0.05 were considered significant, and a standard software package (SAS Institute) was used for analysis. An unpaired t-test was used to determine differences in SZP expression for normal versus OA samples, OA samples with and without detectable blood, and aspirate volume of synovial fluid. P values less than 0.05 were considered significant. The correlation coefficient (r²) was calculated using a second-order polynomial fit of the data.

**RESULTS**

Histologic scores of cartilage damage severity in OA patients were highly correlated with the friction coefficient (r² = 0.999) and SZP expression (r² = 0.819) in different regions of human OA cartilage (Figure 1B).

SZP expression at the medial anterior, medial posterior, and lateral anterior locations was consistently higher than that at the lateral posterior location; however,
statistical differences between SZP expression in the medial anterior, medial posterior, and lateral anterior locations were not observed \( (P > 0.125) \). Samples from medial anterior locations were characterized by significantly higher friction coefficients compared with samples from medial posterior, lateral anterior, and lateral posterior locations \( (P < 0.015) \). In situ human SZP expression patterns and friction coefficients in OA samples demonstrated a dependence on articulating knee joint location and maximum OA score, in contrast to the SZP expression and friction coefficient of samples from normal subjects, which did not show a dependence on maximum OA score but only variation with joint location (Figure 1C).

OA severity showed a dependence on knee joint location (Figure 1B). The maximum OA score was found at the medial anterior, medial posterior, lateral anterior, and lateral posterior locations in 17 (81.0%), 3 (14.3%), 1 (4.8%), and 0 (0.0%), respectively, of all OA subjects \( (n = 21) \). On average, the medial anterior location demonstrated significantly higher OA scores compared with the medial posterior, lateral anterior, and lateral posterior locations \( (P < 0.011) \). The OA scores in normal subjects were lower on average than those of similar locations in OA subjects (Figure 1C).

SZP expression was observed in all samples at the articular surface regardless of OA severity (Figure 2). Histochemistry (H&E staining) revealed cell and proteoglycan patterns throughout the cartilage thickness that depended on OA severity. In normal subjects, the depth of SZP expression was typically greater in the medial anterior region of the medial condyle (Figures 2A and C).

SZP expression was detected in the synovial fluid of all subjects (Figure 3). Both the SZP expression and the aspirate volume of synovial fluid were significantly elevated in OA patients \( (mean \pm SEM 150.81 \pm 23.08 \mu g/ml and 3.82 \pm 0.80 ml, respectively) \) compared with normal subjects \( (34.82 \pm 27.55 \mu g/ml and 0.98 \pm 0.06 ml, respectively) \) \( (P = 0.048 \) for SZP expression and \( P = 0.002 \) for the aspirate volume of synovial fluid). Blood was visually observed in 52.6% of the OA samples collected. Although not significant \( (P = 0.110) \), a mean difference was noted between OA samples with and those without a detectable presence of blood (Figure 3A). The mAb S6.79 recognized the large \( (~345-kd) \) PRG4 gene product (Figure 3B). The levels of the cytokines TNF\( \alpha \) and IL-1\( \beta \) were elevated in the synovial fluid of some of the OA subjects (Figure 3C).

**DISCUSSION**

The objective of this study was to determine the localization and concentration of SZP in the articular cartilage and synovial fluid of patients with advanced OA. In addition, the relationship of SZP content with the articular surface friction coefficient, OA severity, and levels of proinflammatory cytokines was examined. The main findings of this study can be summarized as follows: OA severity at different regions of human cartilage correlated with the coefficient of friction, SZP expression patterns and friction coefficient showed a
dependence on articulating knee joint location and maximum OA score, SZP expression was observed in all samples at the articular surface regardless of OA severity, and high SZP expression was detected in the synovial fluid of all subjects with advanced OA.

Regional SZP expression patterns indicated a coupling between biochemical and mechanical function in which physical forces regulate OA severity and joint lubrication through mechanically stimulated secretion of SZP. Particularly for the lateral condyle, for which the maximum OA score was relatively low compared with that of the medial condyle (Figure 1B), higher SZP expression was observed in the load-bearing region (i.e., higher SZP expression in lateral anterior compared with lateral posterior locations). Thus, it appears that the physical forces from typical joint contact resulted in increased chondrocyte mechanotransduction, as previously described (3) and as confirmed by the immunohistochemistry data obtained in samples from normal human subjects (Figures 2A and C). It was not possible to confirm this result for the medial condyle in OA samples because the missing cells and tissue of the superficial zone due to advanced OA precluded a complete analysis.

A surprising finding was the immunolocalization of SZP in the medial condyle despite the high OA score reflecting the loss of superficial zone cells producing the protein (Figure 2B). The fact that SZP was observed in all of the samples can be explained by the increased SZP expression in the synovial fluid (with additionally increased intraarticular volumes), suggesting that SZP/lubricin in synovial fluid served as a boundary-lubricant reservoir for all surfaces lining the synovial joint. Furthermore, it may be that there are increased binding sites in the damaged tissue structure due to the loss of proteoglycans during OA progression. These interpretations are supported by SZP immunolocalization results in samples representative of severe and minimal OA (Figure 2B). Additionally, the presence of blood in some synovial fluid samples may have been indicative of the harvest procedure and may have led to an underestimation of the overall concentration of SZP (Figure 3C).

Reduced concentrations of lubricin and markers of joint inflammation have previously been observed in the synovial fluid of patients within 32–364 days after anterior cruciate ligament injury (5), in contrast to the present study, which showed increased SZP expression and levels of markers of joint inflammation in some, but not all, patients with late-stage OA (Figure 3). The results of the present study suggest an effect of OA stage on SZP/lubricin expression, with a possible rebound and increased SZP expression encountered in the late stage of the disease. However, it is important to consider that the 345-kd PRG4 gene product was consistently observed using mAb S6.79 (Figure 3B), and thus, it is unclear how other gene products varied as a function of location and degree of OA severity. Further, because the samples from normal subjects were obtained postmortem, it is unknown whether SZP levels precisely reflected concentrations in vivo.

SZP (or related biomolecules, such as lubricin) may be ineffective in reducing the friction of boundary-lubricated human joints exhibiting advanced OA because other mechanisms may dominate the tribological response. The friction coefficient increased significantly with the maximum OA score, particularly in the case of medial condyles (medial anterior location), despite relatively similar levels of SZP expression in condyles at the medial posterior and lateral anterior joint locations (Figure 1B). It is thus possible that either cartilage damage causes an increase in the friction coefficient and/or changes in the friction coefficient lead to cartilage damage. This finding has been attributed to other mechanisms that may dominate tribological behavior (11,12). It is well known that the friction coefficient shows a dependence on length scale and operating conditions (e.g., magnitude of contact stresses, apparent contact area, mechanical properties of interacting surfaces, and total sliding distance). For SZP to function properly in the boundary lubrication system, the adsorbed molecular film must be conformal to the surface topography in a closed-packed arrangement. It is believed that the increased surface roughness and variable tissue stiffness in patients with late-stage OA (13) intensified the local stresses at asperity contacts, resulting in the rapid removal of the boundary lubricant and preventing its timely replenishment. The higher friction coefficients of OA samples (particularly for the load-bearing medial anterior location) compared with samples from normal subjects indicate that the sliding conditions were not conducive to the maintenance and/or timely replenishment of a continuous and conformal boundary-lubricating layer. This may be attributed to the increased surface roughness of the OA samples (especially those characterized by a high maximum OA score), which promoted the dominance of other friction mechanisms, such as asperity deformation, adhesion, and plowing. These mechanisms are generally characterized by higher energy dissipation (friction) during sliding as compared with shearing of a boundary-lubricant layer, which dominated the friction behavior of the smoother samples from normal subjects.
Moreover, it has been documented that although synovial fluid from patients with advanced OA lacks superficial zone chondrocytes (in some tissue regions), it maintains normal superficial zone lubricating ability in vitro, indicating that synovial fibroblasts contribute to joint lubrication through lubricin synthesis (14). Considering that the SZP/lubricin detected in the present study may possess normal lubricating ability (14), it is unclear to what extent recombinant lubricin will effectively treat OA (15), particularly when diagnosed in the late stage.

A strong correlation of SZP expression and friction coefficient with maximum OA score was found for OA samples ($r^2 > 0.819$) (Figure 1B), despite the variability inherent in this patient population. A sufficiently large sample size of OA patients ($n = 21$) allowed meaningful comparisons and tests to be conducted; however, such comparisons with normal samples were difficult because of the small sample size of normal subjects ($n = 2$).

Although the principal objective of this study was to document SZP expression patterns in OA patients, it was also of interest to determine whether the regional patterns found in young bovine joints (3) were also present in normal human samples. Patterns similar to those observed in a previous study (3) were revealed by immunohistochemistry in normal human samples (Figures 2A and C), where a greater depth of SZP expression in tissue samples was observed in anterior (particularly medial) than posterior locations. Such patterns may be the result of an in vivo regulation effect of physical forces and joint contact typical of normal physiological activities, a concept reinforced by the OA severity in the medial anterior regions observed in 81% of the OA patients examined. However, ELISA data for normal SZP expression (Figure 1C) did not correspond to the observed immunohistochemistry patterns. This disparity can be attributed to the small sample size ($n = 2$) and to the fact that repeated testing of only these samples by ELISA yielded highly variable values. Thus, the data for normal SZP expression shown in Figure 1C should be interpreted with caution because further studies are necessary to provide a more comprehensive understanding of regional SZP expression in normal human samples. Additionally, because a human SZP standard was not available, a bovine standard was used and may have altered the magnitude of SZP concentration overall.

Structure–function relationships abound in biological systems, such as Wolff’s law in bone (16), and the functional significance of single proteins is well documented in nature (17). The results of the present study reveal a coupling between biochemical and mechanical function in which physical forces regulate OA severity and joint lubrication through the development of SZP in articular cartilage by mechanotransduction. To our knowledge, this is the first study to demonstrate that the mechanical measurement of the friction coefficient correlates so highly with the histologic assessment of cartilage damage in OA patients.

The findings of this study suggest that SZP (or similar biomolecules) may be ineffective in reducing friction under boundary lubrication conditions in human joints at advanced stages of OA, where other mechanisms may dominate the tribological response. Although SZP expression may be mediated by TGFβRII signaling (3), the precise mechanotransduction pathways by which mechanical signals regulate SZP expression and the mechanisms by which SZP lubricates human synovial joints over the lifetime of the organism require further investigation. Such regulatory pathways may provide insight into the progression of cartilage degeneration and regenerative therapies aimed at reconstitution and maintenance of effective boundary lubrication of articular cartilage prior to advanced OA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Neu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design. Neu, Reddi, Komvopoulos, Di Cesare. Acquisition of data. Neu, Schmid, Di Cesare. Analysis and interpretation of data. Neu, Reddi, Komvopoulos, Schmid, Di Cesare.

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