Effects of an articular cartilage lubrication with a viscosupplement in vitro and in vivo following osteochondral fractures in horses

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OBJECTIVE
To assess whether the combination of hyaluronan, sodium chondroitin sulfate, and *N*-acetyl-D-glucosamine (HCSG) lubricates articular cartilage in vitro and modulates joint lubrication in vivo.

ANIMALS
16 healthy adult horses.

PROCEDURES
The effects of HCSG injections on SF lubricant properties and joint health, immediately after injury and 2 weeks later, were analyzed by use an equine osteochondral fracture model of post-traumatic osteoarthritis (OA). Middle carpal joints of adult horses were randomly assigned to 1 of 4 surgical treatment groups as follows: normal nonsurgical group (n = 8), normal sham-surgical group (8), OA-induced surgical group with HCSG injection (8), or OA-induced surgical group with saline (0.9% NaCl) solution injection (8). Synovial fluid was aspirated periodically and analyzed for boundary lubrication function and lubricant molecules. At 17 days, joints were screened for gross pathological changes.

RESULTS
Induction of OA led to an impairment of SF lubrication function and diminished hyaluronan concentration in a time-dependent manner following surgery, with HCSG injection lessening these effects. Certain friction coefficients approached those of unaffected normal equine SF. Induction of OA also caused synovial hemorrhage at 17 days, which was lower in joints treated with HCSG.

CONCLUSIONS AND CLINICAL RELEVANCE
After induction of OA, equine SF lubricant function was impaired. Hyaluronan-sodium chondroitin sulfate–*N*-acetyl-D-glucosamine injection restored lubricant properties at certain time points and reduced pathological joint changes. (*Am J Vet Res* 2021;82:611–618)

One of the functions of articular cartilage and SF, working together, is to lubricate joints during motion. Joint injury[1–3] and joint surgery[4] result in reduction of SF lubricant properties in association with variable and time-dependent decreases in the concentration of high molecular weight HA and changes in the concentration of PRG4 lubricants. Viscosupplementation by injection of lubricating fluids into the joint may provide pain relief and chondroprotection, in part, because of restoration of lubrication in injured or diseased joints.[1]

ABBREVIATIONS

CS	Chondroitin sulfate
GAG	Glycosaminoglycan
HA	Hyaluronan
HCSG	Hyaluronan-sodium chondroitin sulfate–*N*-acetyl-D-glucosamine
IACUC	Institutional Animal Care and Use Committee
NAG	*N*-acetyl-D-glucosamine
OA	Osteoarthritis
OCF	Osteochondral fracture
PRG4	Proteoglycan-4
SF	Synovial fluid

The combination of HA, CS, and NAG (ie, HCSG) has been developed and used as a supplement or replacement of SF following an injury or surgery. Hyaluronan-sodium chondroitin sulfate–*N*-acetyl-D-glucosamine use is indicated in veterinary medicine for postsurgical lavage of the synovial space.[5] The HA is of high molecular weight (750 kDa) and provided at a concentration of 5 mg/mL. Chondroitin sulfate has a molecular weight of approximately 15 kDa and is provided at a high concentration[6] (100 mg/mL), as is NAG (100 mg/mL).

Hyaluronan-sodium chondroitin sulfate–*N*-acetyl-D-glucosamine has chondroprotective effects in vivo in experimentally induced OA and in vitro in equine impact damaged articular cartilage. Intra-articular injection of HCSG in the equine OCF model of post-traumatic OA (which induces clinical, gross, histologic, and biochemical changes indicative of OA)[5,7] reduced lameness, full-thickness cartilage erosion, and radiographic evidence of boney proliferation at the joint margins at 10 weeks, relative to place-

bo-injected joints with OCF.[5] Also, HCSG treatment of cartilage explants following impact loading reduced cell death, compared with impacted explants without HCSG treatment.[8]

The purposes of the present study were to assess whether HCSG lubricates articular cartilage in vitro and modulates joint lubrication in vivo. The hypothesis was that, because HCSG is a combination of lubricant constituents (HA and CS) that have been shown individually to lubricate cartilage in vitro,[1,9,10] injection of HCSG into a joint after injury may improve cartilage lubricant composition, lubrication function, and joint health. The specific objective was to determine the ability of HCSG injection into the injured joint of the equine OCF model of post-traumatic OA to enhance SF lubricant properties at various times after intra-articular injection.

Materials and Methods

Study of the lubricating effects of HCSG

Under the auspices of the study protocol approved by the Colorado State University IACUC, the time-dependent effects on SF lubricant components and lubrication function of HCSG[a] injection into joints following induction of OA were evaluated in the present study by use of the equine OCF model of post-traumatic OA.[5,7] Because a previous study[5] showed the development of OA at 70 days in the equine OCF model, it was the concern of the IACUC that a nonterminal study may have adverse long-term consequences in the study horses. Thus, the IACUC approved the protocol for a terminal study, ending after 17 days.

Injection at the time of injury and 2 weeks after injury with additional sample collection times were chosen to assess all joint lubricant changes, acknowledging the short residence time of injected HA following injury.[11,12] Clinical use of HCSG, as initially recommended by the manufacturer,[a] is for 4 weekly injections but the present study was primarily conducted to assess changes in SF.

Sixteen healthy adult horses, with a mean ± SD age of 3.2 ± 0.4 years (range, 2.0 to 5.0 years), 10 female and 6 male, weighing 378 ± 19 kg with a range of 290 to 445 kg, were obtained for the study and underwent surgery on one or both middle carpal joints. Before and after joint manipulation, horses were in good health, without palpable effusions or radiographic abnormalities, and free of lameness. Prior to the onset of the study, horses underwent an orthopedic assessment by a board-certified veterinary surgeon and findings were within normal limits.

Middle carpal joints of adult horses were randomly assigned to 1 of 4 surgical treatment groups as follows: normal nonsurgical group (NL group, n = 8), normal sham-surgical group (sham group, 8), OA-induced surgical group with HCSG injection (OA-HCSG group, 8), or OA-induced surgical group with saline (0.9% NaCl) solution injection (OA-saline group, 8). Synovial fluid samples were obtained just before (time 0–) and after (time 0+) surgery and injection; at 4 hours, 1 day, and 3 days after surgery; just before (day 14-time 0–) and after (day 14-time 0+) a second injection at 14 days; and at 4 hours (day 14 + 4 hours), 1 day (15 days), and 3 days (17 days) following the second injection. Joints were evaluated for gross pathological changes and SF was analyzed for biochemical properties and lubricant function.

For each horse, one joint was randomly assigned to either the NL group (n = 8 joints) or sham group (8) and the opposite joint underwent OCF induction of OA disease[5] and received 5-mL intra-articular injections of either saline solution (OA-saline group, 8) or HCSG (OA-HCSG group, 8) just after surgery (after recovery from anesthesia for surgery and after sterile preparation of the carpus) or at 14 days after surgery. In OA joints, an osteochondral fragment with bone debris was created as described previously.[5,13–16] Following surgery, each horse was housed in a stall (3.65 × 3.65 m). Beginning on day 10, horses were exercised 6 min/d divided sequentially into 2 minutes trot, 2 minutes gallop, and 2 minutes trot on a high-speed treadmill.

From each middle carpal joint, approximately 4 mL of SF was aspirated immediately prior to (approx 15 minutes) surgery (time 0–), at 14 days just before the second injection (day 14-time 0–), and at the termination of the study (17 days, **Figure 1**). The injection time points for HCSG or saline solution were selected on the basis of the recommended clinical use of HCSG. From each middle carpal joint, approximately 0.8 mL of SF was aspirated from each joint at additional time points as follows: approximately 10 minutes after surgery and injection (time 0+); at 4 hours, 1 day, and 3 days after surgery; and at approximately 10 minutes (day 14-time 0+), 4 hours (day 14 + 4 hours), 1 day (ie, 15 days after surgery), and 3 days (ie, 17 days after surgery) after the second injection. These time points for joint sample collection after surgery were selected to determine the time-dependent change in HCSG content in the joint on the basis of the known rapid clearance of HA from diseased joints[11] and the short residence time (6 hours) of HA following HA injection in previous animal studies of joint injury.[12] Synovial fluid was aspirated by use of a standard 18-gauge hollow bore needle attached to a 10-mL syringe and clarified of cells and debris by centrifugation at 15,000 × *g* for 30 minutes at 4°C. The supernatant was stored at –70°C until use.

Synovial fluid lubricant properties were assessed by biochemical analysis of SF samples for concentrations of protein, HA (primary outcome), PRG4, and GAG, as well as for HA molecular weight distribution, and by friction (secondary outcome) tests in the boundary mode of lubrication. Because of the limited volume, SF samples from NL and sham group joints were only tested for friction properties at time 0–, day 14-time 0–, and 17 days. For friction tests, normal adult bovine articular cartilage substrate pairs

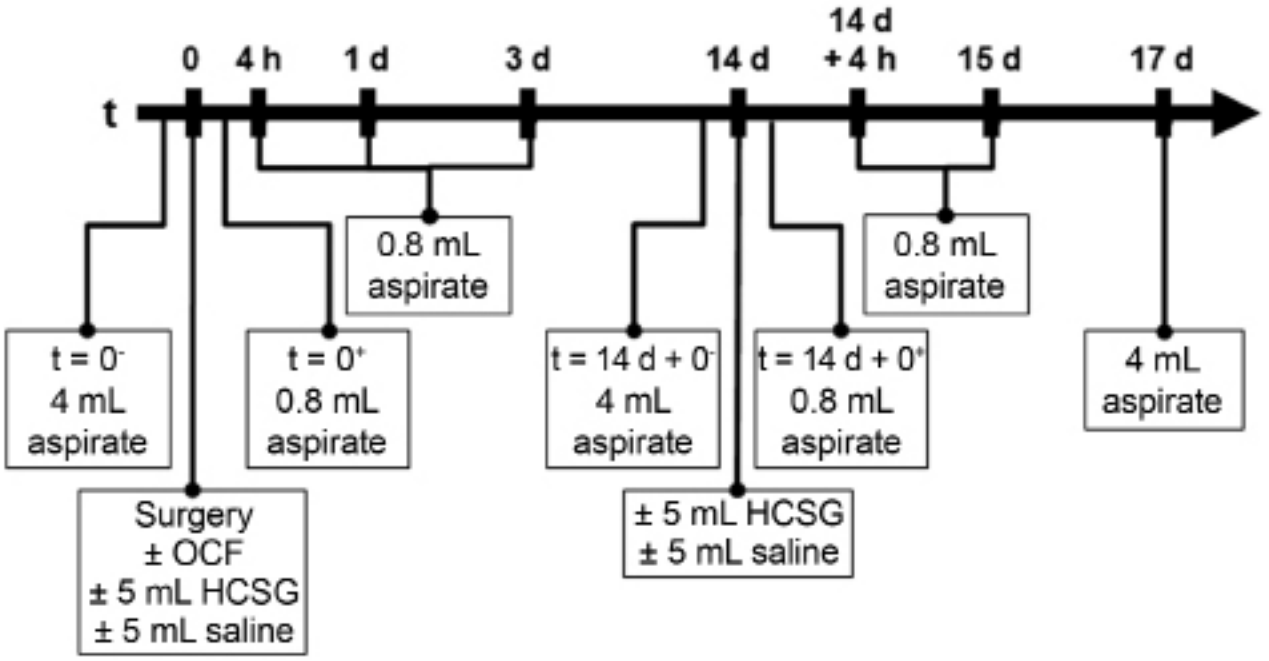

Figure 1—Research study timeline. Briefly, 16 healthy adult horses underwent surgery on 1 or both middle carpal joints. The middle carpal joints were randomly assigned to 1 of 4 surgical groups as follows: normal nonsurgical group (NL group, n = 8); normal sham-surgical group (sham group, 8); OA-induced surgical group with HCSG injection (OA-HCSG, 8); or OA-induced surgical group with saline (0.9% NaCl) solution injection (OA-saline group, 8). From each middle carpal joint, approximately 4 mL of SF was aspirated immediately prior to (approx 15 minutes) surgery (time 0−), at 14 days just before the second injection (day 14-time 0−), and at the termination of the study (17 days). In addition, 0.8 mL of SF was aspirated from each joint at the following time points: approximately 10 minutes after surgery and injection (time 0+); at 4 hours, 1 day, and 3 days after surgery; and at approximately 10 minutes (day 14-time 0+), 4 hours (day 14 + 4 hours), 1 day (ie, 15 days after surgery), and 3 days (ie, 17 days after surgery) after the second injection.

were tested sequentially in PBS solution (test A) and then in 3 SF samples (tests B, C, and D) from the same time point and same treatment group, in a random sequence. Equine SF portions were assayed as indicated, typically in duplicate, until the coefficient of variation was < 15%. Proteoglycan-4 was assayed by Western blot with an anti-lubricin antibody[1,b] after digestion with *Streptomyces hyaluronidase*[c] (1 U/mL, 37°C overnight), digitization, and comparison to an equine PRG4 standard.[17] Glycosaminoglycan was also assayed after hyaluronidase digestion by use of dye binding with a shark CS standard.[18,d] Hyaluronan was quantified by an ELISA-like assay after digestion with proteinase K[e] (0.5 mg/mL; 37°C overnight) and boiling for 10 minutes.

Solutions were tested for friction-reducing boundary mode lubrication properties on normal articular cartilage as described previously.[10] The concentration-dependent cartilage lubricating function of HCSG and its constituent components were assessed in separate studies (**Supplementary Appendix S1**, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.8.611). Osteochondral substrates were isolated from the patellofemoral groove of adult bovine stifle joints,[1,4,10] rinsed over 36 hours in PBS solution at 4°C to remove residual SF,[4,10] equilibrated overnight at 4°C in 0.5 to 1 mL of lubricant solution, and then tested in that lubricant solution.[10] In lubrication tests, as done previously,[19] up to 5 lubricant solutions were tested over consecutive days on the same grossly normal bovine osteochondral substrate sample pair. Substrate pairs were maintained under bathing and testing conditions that maintained substrate integrity but allowed detection of altered bathing fluid lubricant properties.[19] Depending on the study, the sequential test solutions either had increasing concentration of putative defined lubricant or were from the same in vivo experimental group. Osteochondral pairs were placed in apposition in lubricant solutions and subjected to 18% cartilage compression, an effective sliding velocity of 0.3 mm/s, and prespin pause times of 1.2, 12, and 120 seconds. Friction coefficients were calculated by use of the equilibrium axial load following a 30-minute stress relaxation. A static friction coefficient was calculated by use of the peak torque measured within the first 10° of the start of rotation and after the 120 seconds prespin pause. A kinetic friction coefficient was calculated from an averaged torque during steady-state sliding. The kinetic friction coefficient data are presented as the average value for all prespin pauses, consistent with previous studies, because the kinetic friction coefficient does not vary substantially with prespin pause times.[1,19]

At day 17, horses were euthanized by IV injection of pentobarbital sodium solution (390 mg/mL) at a dose of 0.275 mL/kg. Gross pathological assessment of each middle carpal joint was performed to screen for cartilage and synovium disease with a gross morphological grading system, developed in 2002[13] and subsequently detailed in 2010.[20] An experienced individual evaluator (DDF), blinded to the treatment assignment, used a macroscopic scoring system of total erosion on an ordinal scale from 0 to 4 (0 = no gross fibrillation or fissuring, 1 = very superficial erosion with articular cartilage swelling, 2 = partial thickness erosion, 3 = partial- and full-thickness erosion, and 4 = extensive full-thickness erosion to the level of subchondral bone). The condition of the synovium was evaluated separately for hypertrophy, hemorrhage, and inflammation by use of a scale from 0 to 4, where 0 represents normal appearance and 4 represents severe pathological change. There were no adverse events during the study.

Statistical analysis

Power analysis for the primary outcome, HA concentration, resulted in 8 joints/treatment condition for a total of 16 horses. In particular, planning for a treatment effect of 0.6 mg/mL and an SD of 0.3 mg/mL (based on previous studies of the time course of diminution of HA concentration following experimental injury[12] and measured HA concentrations in horses[1,4]) yielded 6 horses; 2 additional animals/group were used to provide increased power.

Normality[f] was checked by use of the Shapiro-Wilk test, and homogeneity of variances was assessed by use of the Levene test. To assess normally distributed and homoscedastic data, data at time 0+, 4 hours, day 14-time 0+, and day 14 + 4 hours, were log transformed. The effect of treatment groups (NL, sham, OA-saline, OA-HCSG) and time on concentrations of protein, PRG4, CS, and HA was assessed by a mixed model ANOVA with animal as a random factor followed at each time point by 1-way ANOVA with a fixed factor of treatment and random effect of animal and post hoc Tukey tests. The effect of time on the friction properties of SF samples from NL group joints was assessed by mixed model ANOVA with time (ie, time 0–, day 14-time 0–, and 17 days) as the main effect and animal as the repeated factor. The effects of treatment and time on friction properties were assessed by a mixed model ANOVA with animal as a random factor, followed by an assessment of the effects of treatment at each time point on friction properties by 1-way ANOVA, followed by post hoc Tukey tests, with friction properties at time 0–, 4 hours, 1 day, and 3 days, compared with those of SF samples from NL group joints at time 0–, day 14-time 0–, day 14-time 0+, day 14 + 4 hours; friction properties at 15 days, compared with SF samples from NL group joints at day 14-time 0–; and friction properties at 17 days, compared with SF samples from NL group joints at 17 days. The effect of treatment group on gross pathological scores was assessed by Kruskal-Wallis nonparametric tests followed by Dwass-Steel-Critchlow-Fligner pairwise comparisons.[21] Relationships between gross pathological scores and HA concentrations were assessed by Spearman rank correlation.

All data are reported as mean ± SEM. For certain experimental groups and time points in the in vivo experiment, there was insufficient SF fluid volume for all of the tests and the resultant number of samples was < 8, where indicated. Values of $P < 0.05$ were considered significant.

Results

Postmortem examination

Joint tissues from OA-HCSG joints had certain gross pathological scores that were significantly lower than OA-saline joints. Joints that underwent surgery (irrespective of sham or OA joints) showed evidence of synovial hemorrhage and were significantly ($P < 0.05$) affected by treatment group **(Figure 2)**. Relative to NL joints, synovial hemorrhage score was higher for sham (117 ± 43%; $P < 0.05$) and OA-HCSG (100 ± 38%; $P < 0.01$) joints and highest for OA-saline joints (283 ± 94%; $P < 0.01$). The OA-HCSG joints had a lower (–48 ± 11%; $P < 0.01$) synovial hemorrhage score than OA-saline joints.

Erosion scores were higher in joints in which OA was induced. Total (Figure 2) erosion scores were significantly ($P < 0.005$) affected by treatment group. Relative to NL joints, the total erosion score was similar for sham joints ($P = 0.46$) and was significantly higher for OA-HCSG (550 ± 280%; $P < 0.05$) and OA-saline (700 ± 360%; $P < 0.01$) joints. In summary, pathological scores were higher for joints where OA was induced, with protection from synovial hemorrhage being offered by treatment with HCSG.

HA concentration

Synovial fluid HA concentrations of NL joints fluctuated slightly during the 10 time points with a mean of 0.62 ± 0.05 mg/mL (range, 0.37 to 0.82 mg/mL; Supplementary Appendix S1). Synovial fluid HA concentrations were affected by treatment ($P < 0.005$) and time ($P < 0.005$) as well as by treatment and time interactively ($P < 0.005$). Synovial fluid HA concentrations varied significantly between treatment groups at time 0+, 4 hours, day 14-time 0+, and day 14 + 4 hours. synovial fluid HA concentrations of sham joints were significantly (–54 ± 16%; $P < 0.05$) lower than those of NL joints at 4 hours after surgery. In contrast, synovial fluid HA concentrations of OA-HCSG joints were significantly higher than those of NL joints, just after each injection at time 0+ (434 ± 167%; $P < 0.05$), at day 14-time 0+ (1,655 ± 250%; $P < 0.001$), and also 4 hours after surgery (111 ± 27%; $P < 0.05$). Relative to that of OA-saline joints, SF HA concentrations in OA-HCSG joints were significantly higher at time 0+ (26,900 ± 8,160%; $P < 0.001$), day 14-time 0+ (5,400 ± 1,600%; $P < 0.001$), 4 hours after

injection (60 3 ± 133%; $P < 0.001$), and day 14 + 4 hours (242 ± 86%; $P < 0.001$). Generally, SF HA concentrations in OA-HCSG joints was significantly higher for ≥ 4 hours following surgery and injections. The molecular weight distribution of HA in SF samples was assessed (Supplementary Appendix S1).

GAG concentration

Synovial fluid GAG concentrations of NL joints varied slightly during the 10 time points with a mean of 0.067 ± 0.01 mg/mL (range, 0.03 to 0.13 mg/mL; Supplementary Appendix S1). Synovial fluid GAG concentrations were significantly dependent on treatment ($P < 0.005$), time ($P < 0.005$), and treatment and time interactively ($P < 0.005$). Synovial fluid GAG concentrations varied between groups at time 0+, 4 hours after surgery, day 14-time 0+, day 14 + 4 hours, and 15 days after surgery. Synovial fluid GAG concentrations of sham and OA-saline joints were similar to NL joints at all time points ($P = 0.052$ to 1.00). Synovial fluid GAG concentrations of OA-HCSG joints, relative to NL joints, were significantly higher just after each injection, at time 0+ (30 ± 5 mg/mL; $P < 0.001$) and at day 14-time 0+ (70 ± 8 mg/mL; $P < 0.001$), and 4 hours following each injection (2.2 ± 0.7 mg/mL at 4 hours [$P < 0.005$] and 0.9 ± 0.2 mg/mL at day 14 + 4 hours [$P < 0.001$]). Synovial fluid GAG concentrations were slightly higher in sham joints than OA-saline and OA-HCSG joints. Similar to HA, increased SF GAG concentration persisted in the joints for ≥ 4 hours following surgery and injections.

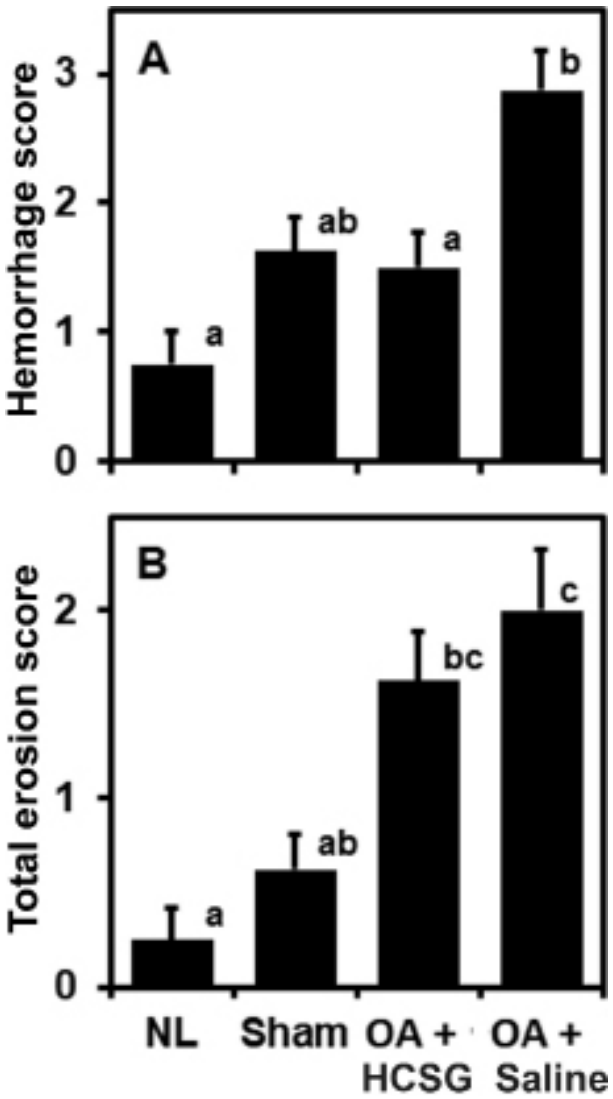

Figure 2—Gross pathological scores for equine middle carpal joints that were either NL group joints, sham group joints, or had OA induced and received injections just after surgery (time 0+) or at 14 days after surgery (day 14-time 0+) of HCSG (OA-HCSG group joints) or saline solution (OA-saline group joints). At the end of the study (time 17 days), joints were graded for the degree of (panel A) synovial membrane hemorrhage on a scale of 0 (normal) to 4 (severe) and (panel B) total erosion score on a scale of 0 (normal) to 4 (severe change). Differing letters indicate significant differences between groups at $P < 0.05$ (mean ± SEM; n = 6 to 8 samples/group).

PRG4 concentration

Equine SF PRG4 concentrations of NL joints fluctuated slightly during the 10 time points with a mean of 64 ± 12 µg/mL (range, 10 to 96 µg/mL; Supplementary Appendix S1). Synovial fluid PRG4 concentrations were significantly dependent on treatment ($P < 0.01$), time ($P < 0.005$), and treatment and time interactively ($P < 0.01$). Synovial fluid PRG4 concentration varied between treatment groups at 4 hours, 1 day, day 14 + 4 hours, and 17 days after surgery. Compared with NL joints, sham joints had higher SF PRG4 concentrations at 4 hours (239 ± 95%; $P < 0.005$) and 1 day (92 ± 35%; $P < 0.05$) after surgery. At day 14 + 4 hours, SF PRG4 concentrations were 2.2 to 2.4 times as high in OA-saline and OA-HCSG joints than in NL joints ($P < 0.05$) and were 1.9 times as high in OA-HCSG joints than sham joints ($P < 0.05$). At 17 days after surgery, SF PRG4 concentrations were 2 times as high in OA-saline and OA-HCSG joints than sham joints ($P < 0.05$).

Friction coefficients

At time 0+, 1 day, 3 days, and day 14-time 0+, the static and kinetic friction coefficients were elevated after surgery and injection.

Lubricant function of equine SF was consistent in healthy joints, impaired by injury, and partially restored by injection of HCSG (Supplementary Appendix S1). Consistent with removal of residual SF from the articular surface, the static friction coefficient was 0.35 ± 0.01 and the kinetic friction coefficient was 0.20 ± 0.01 for substrates tested in PBS solution. The static and kinetic friction coefficients of SF from the healthy joints prior to surgery were similar between groups ($P = 0.85$ and $P = 0.18$, respectively) with means of 0.097 ± 0.02 and 0.031 ± 0.002, respectively, and those of NL joints varied only slightly during the course of the study.

Static friction was not dependent on treatment alone ($P = 0.11$), but it was significantly dependent on time ($P < 0.005$) and interactively dependent on treat-

ment and time ($P < 0.005$; Supplementary Appendix S1). Static friction of equine SF from OA-saline joints, relative to NL joints, were markedly higher just after surgery (176 ± 14%; $P < 0.001$), similar at 4 hours after surgery ($P = 0.31$), higher after surgery at 1 day (35 ± 2%; $P < 0.005$) and 3 days (56 ± 5%; $P < 0.001$), and similar at subsequent time points ($P = 0.65$ to 0.97). Static friction of SF from OA-HCSG joints, relative to NL joints, were slightly higher just after surgery (53 ± 4%; $P < 0.001$), similar at 4 hours ($P = 0.12$) and 1 day ($P = 0.24$) after surgery, higher at 3 days after surgery (42 ± 3%; $P < 0.01$) and day 14-time 0+ (52 ± 8%; $P < 0.05$), and similar at subsequent time points ($P = 0.30$ to 0.85). Relative to that of OA-saline joints, static friction of OA-HCSG joints was lower at time 0+ (−45 ± 4%; $P < 0.001$) and 1 day (−16 ± 1%; $P < 0.05$), higher at day 14-time 0+ (41 ± 7%; $P < 0.05$), and not significantly different at other time points ($P = 0.14$ to 0.98).

Kinetic friction was affected by treatment ($P < 0.005$), time ($P < 0.005$), and interactively by treatment and time ($P < 0.005$). The kinetic friction coefficient (Supplementary Appendix S1) of NL, sham, OA-saline, and OA-HCSG joints was modulated with time in a manner similar to that described for static friction. Kinetic friction of equine SF from OA-saline joints, relative to NL joints, was markedly higher just after surgery (458 ± 23%; $P < 0.001$) and at 4 hours after surgery (91 ± 11%; $P < 0.005$), similar at 1 day ($P = 0.063$), higher at 3 days (93 ± 10%; $P < 0.001$) after surgery, and similar at subsequent time points ($P = 0.11$ to 0.92). Kinetic friction of SF from OA-HCSG joints, relative to NL, was slightly higher at time 0+ (186 ± 15%; $P < 0.005$), 4 hours (54 ± 8%; $P < 0.005$), 1 day (37 ± 5%; $P < 0.005$), and 3 days (38 ± 6%; $P < 0.05$) after surgery; similar just before the second injection ($P = 1.0$); higher just after the second injection (45 ± 7%; $P < 0.005$); and similar at subsequent time points ($P = 0.07$ to 1.0). Relative to that of OA-saline joints, kinetic friction of OA-HCSG joints was lower at time 0+ (−49 ± 6%; $P < 0.001$) and 3 days (−29 ± 6%; $P < 0.005$) and similar at other time points ($P = 0.07$ to 0.54).

Relationships between lubricant concentrations and gross pathological scores

The synovial hemorrhage score was related to SF concentrations of HA and at certain time points. The synovial hemorrhage score decreased with increasing SF HA concentrations at time 0+ ($\rho = -0.53$; $P < 0.005$), 4 hours after surgery ($\rho = -0.49$; $P < 0.01$), day 14-time 0+ ($\rho = -0.38$; $P < 0.05$), and day 14 + 4 hours ($\rho = -0.50$; $P < 0.005$) and with increasing time-averaged SF HA concentration ($\rho = -0.49$; $P < 0.005$). Synovial hemorrhage scores decreased with increasing HA concentrations.

Discussion

Findings of the present study indicated that HCSG can lubricate articular cartilage in vitro and transiently enhance lubrication properties of SF following an injury or surgery. Hyaluronan-sodium chondroitin sulfate–*N*-acetyl-D-glucosamine provided boundary lubrication function at a cartilage-cartilage interface in a dose-dependent manner, with even 10% HCSG lubricating better than PBS alone and with HA and CS contributing to HCSG's lubrication function. In the equine OCF model of OA, HCSG ameliorated the impairment of lubrication function at early time points (time 0+ and 4 hours after surgery) following joint injury and concomitantly elevated HA and CS concentrations in SF. The decrease in synovial hemorrhage and mild joint disease at 17 days after surgery suggests that HCSG may be protective of joint health in a manner that is possibly mediated by a variety of mechanical and biological mechanisms.

The in vivo study design had advantages but also some limitations. Saline solution injection served as a control for the injection of HCSG. Although such injection may have diluted SF components, it controls for the volume of HCSG injected and mimics an arthroscopic procedure during which SF is washed out with saline solution. The uninjured contralateral joint that did not undergo surgery may not truly represent an unaltered normal joint as the result of systemic effects of surgery or injury but allowed for a bilateral design with matched left and right joints. In addition, the novel approach of multiple aspirations of small quantities of SF from the same joint allowed for comparison at 10 time points for each joint, minimizing the use of animals. Had separate animals been used for each time point, the number of animals would have been 10 times (for each of the time points) as high, or 160, instead of 16. Also, individual animals have much less variability in SF lubricant concentrations than different animals.[4] Additionally, the inclusion of the sham group allowed for assessing the effects of surgery alone. This present study design can help elucidate the lubrication properties of SF in a large animal model of post-traumatic OA.

The concentration of high molecular weight HA appeared to govern the cartilage-on-cartilage friction-lowering boundary lubrication function of HCSG alone. For HCSG and HA alone, static and kinetic friction were similar to those of HA of similar molecular weight (750 kDa),[1] whereas at full strength, lubrication by HCSG was indistinguishable from that of normal equine SF. Normal equine SF had characteristics consistent with previous reports, with static and kinetic friction coefficients (baseline, time 0–) comparable to values for equine SF,[1,4] human SF (0.022),[1,2] and bovine SF (0.021),[10,22] and HA concentration and molecular size similar to equine SF.[1,2,23] At time 0+ and 4 hours after surgery, the elevated HA concentration in equine SF from OA-HCSG group joints in comparison to NL group joints was consistent with HCSG injection. For equine SF from sham group joints at time 0+ and 4 hours after surgery, a slight decrease in HA concentration is typical,[1,2,23] and the lower HA concentration in equine SF of OA-saline group joints may be in part

the result of surgery. At time 0+, the improvement (lowering) of static and kinetic friction of OA-HCSG group joints, compared with OA-saline group joints, similar to that of NL group joints, together with the correlation of lower friction coefficients with higher HA, especially higher molecular weight (2.5 to 7 MDa) HA,[1] supports the role of HA in cartilage lubrication. Hyaluronan may act as a good boundary lubricant by localizing at the tissue surface because of its higher molecular weight and increased entanglement with other surface molecules.[24,25] Other factors in OA joints, including joint effusion, may have contributed variably to altered lubricant composition and function. Although HCSG injection following injury may help sustain low-friction joint articulation in injured joints, its relatively short duration of effects suggests that it, like other HA preparations, may have longer-lasting effects through a variety of mechanisms.[26]

The stable and variably increased SF PRG4 concentration has a number of implications. Following induction of OA, the maintained or increased SF PRG4 concentration relative to that of NL joints was consistent with a recent report[3] and studies[1,2,27–29] following injury. Differences in absolute concentration of PRG4 between the present study and previous studies may be the result of differences in the method of quantification and antibody used, so the differences should be considered as relative from control values.[1–3,27–29] The difference in SF of NL joints likely reflects increased cellular synthesis of PRG4 by chondrocytes or synoviocytes under the influence of chemical[30–33] or mechanical regulatory factors.[34–37] However, immediately after OCF injury and either saline solution or HCSG injection (time 0+, day 14-time 0+), considering SF time constants associated with equilibration,[38] the maintenance of PRG4 concentration may involve tissue reservoirs, such as cartilage matrix,[39] that buffer perturbations to SF. Because the combination of PRG4 and HA can lubricate cartilage better than either alone,[19,40] maintaining and increasing SF PRG4 may be protective to the joint, and additional PRG4 supplementation may be beneficial.

Screening of joints by gross inspection suggested mild cartilage erosion and moderate synovial change induced by OCF being improved by HCSG treatment through a mechanism other than direct chondroprotection. Scores for synovial membrane hemorrhage were significantly lower for OA-HCSG group joints (and sham group joints) than OA-saline group joints in the present 17-day study. Thus, some of the effects of HCSG may have been through the dampening of synovial inflammation following the induction of OA. The inverse correlation between synovial membrane hemorrhage scores and HA concentrations in the SF samples may have reflected a variety of biological effects of HA. The correlation of reduced synovial inflammation with higher concentrations of HA supported results of previous studies of HA anti-inflammatory and analgesic effects.[41,42]

Taken together, the findings that HCSG lubricates at a cartilage-on-cartilage interface and that injection of HCSG into the joint can enhance equine SF lubricant function following OCF suggested that intra-articular lubricant supplementation may help maintain and restore the boundary lubrication function of SF following surgery or injury. Although the largest variation in lubricant function occurs in the first 4 hours following surgery or injury, the time-dependent variation in lubricant function of equine SF from OA-HCSG and OA-saline group joints, and the relatively short residence time of HA,[12] suggested that repeated applications or extended-release formulations may be beneficial. Thus, SF supplementation with HCSG following a joint injury or repair can transiently restore the lubricating function of SF and may lessen pathological joint deterioration after injury.

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Footnotes

a. Polyglycan, Arthrodynamic Technologies, Lexington, Ky.
b. Abcam, Cambridge, England.
c. Hyaluronidase, MP Biomedical, Irvine, Calif.
d. Chondroitin 6-sulfate sodium salt, Sigma-Aldrich, St Louis, Mo.
e. Roche Applied Science, Penzberg, Bavaria, Germany
f. Systat, version 13, Systat Software Inc, San Jose, Calif.

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