A Synthetic Bottle-brush Polyelectrolyte Reduces Friction and Wear of Intact and Previously Worn Cartilage

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Abstract

A poly(7-oxanorbornene-2-carboxylate) polymer containing pendent triethyleneglycol (TEG) chains of 2.8 MDa ("2.8M TEG") was synthesized and evaluated for long-term lubrication and wear reduction of ex vivo bovine cartilage as well as for synovitis in rats and dogs after intra-articular administration. Bovine cartilage surfaces were tested under torsional friction for 10,080

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CONTRIBUTIONS

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M. Wathier contributed to the experiment design, data interpretations, and review of the manuscript.

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J.D. Freedman contributed to the experiments, data interpretations, and review of the manuscript.

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B.D. Snyder contributed to the experiment design, data interpretations, data analysis, and manuscript writing as well as resources to perform the experiments.

SUPPORTING INFORMATION AVAILABLE

The Supporting Information (SI) document is available free of charge and includes greater experimental details on: the synthesis of the polymer, the contrast-enhanced μCT imaging, the baseline coefficient of friction (μ) testing, the analysis of GAG content in the wear solutions, the safety and gait analysis of the male Lewis rats, and acute safety study in beagles.

COMPETING INTERESTS

M.W.G., M.W., and B.D.S. had a conflict of interest, as they owned shares in FlexBiomedical, Inc, a company that specializes in the design and development of biolubricants for OA treatment, as well as have grant funding from the NIH and Coulter Foundation.

rotations while immersed in either saline, bovine synovial fluid (BSF), or 2.8M TEG. For each solution, coefficient of friction (μ), changes in surface roughness, and lost cartilage glycosaminoglycan were compared. To directly compare 2.8M TEG and BSF, additional samples were tested sequentially in BSF, BSF, 2.8M TEG, and then BSF. Finally, another set of samples were tested twice in saline to induce surface roughness and then tested in BSF, Synvisc, or 2.8M TEG to determine each treatment's effect on worn cartilage. Next, male Lewis rats were injected in one knee with 2.8M TEG or saline and evaluated for effects on gait, and female beagles were injected with either 2.8M TEG or saline in one knee, and their synovial tissues analyzed for inflammation by H&E staining. Treatment with 2.8M TEG lowers μ, lessens surface roughness, and minimizes glycosaminoglycan loss compared to saline. The 2.8M TEG also reduces μ compared to BSF in pairwise testing and on worn cartilage surfaces. Injection of 2.8M TEG in rat or beagle knees gives comparable effects to treatment with saline, and does not cause significant synovitis.

Graphical Abstract
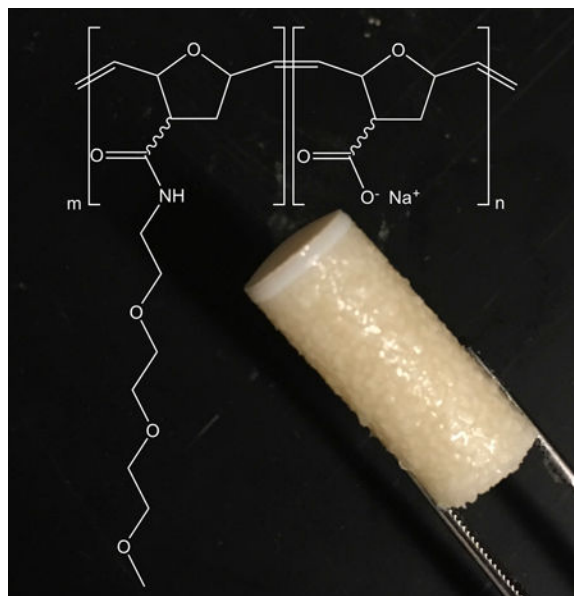
Keywords

biolubricant; synovial fluid; viscosupplement; chondroprotection; osteoarthritis; CT imaging

INTRODUCTION

In healthy diarthrodial joints, articular cartilage and synovial fluid (SF) maintain a nearly frictionless surface that supports and dissipates applied loads. Specifically, interstitial fluid exudation from cartilage surfaces enables fluid-enhanced lubrication during locomotion by creating a fluid film between apposing cartilage surfaces in the articulating joint. Further, the SF constituents hyaluronic acid (HA) and lubricin maintain low friction by preventing direct contact between the apposing cartilage surfaces. During early osteoarthritis (OA) the

hydraulic permeability of cartilage increases due to loss of glycosaminoglycans (GAGs)[1–3] resulting in more rapid fluid excretion during joint movements and diminished fluid-film lubrication. Therefore, more of the friction is born by the extracellular matrix surface. This occurs simultaneously with decreases in the concentrations of lubricin[4] and HA[5], affording early surface damage to the collagen network. This damage decreases cartilage tensile stiffness initially at the surface, but eventually progresses to the deeper layers with a reduction of cartilage mechanical integrity[6–8], thinning of the cartilage tissue, and formation of cartilage lesions. The higher frictional loading[9] and severe wearing[10] eventually erode the cartilage surfaces, and movement can become painful as bone-on-bone contact occurs. Therefore, early OA treatments that reduce excessive cartilage wear would be beneficial.

Current OA treatments range in complexity and invasiveness depending on the severity of the disease. Typical treatments for early-stage OA include weight loss, exercise, activity modification, assistive devices (e.g., canes), and non-steroidal anti-inflammatory drugs. When these methods are unsuccessful or the disease progresses, prescription anti-inflammatory drugs (e.g., intraarticular corticosteroids) or surgical techniques (including partial or total joint arthroplasty) are required. Joint arthroplasty is typically considered a last resort for patients and is the most invasive. Microfracture, osteochondral transfer, and autologous chondrocyte implantation are less invasive and are moderately effective at treating cartilage focal defects of up to 1 cm^2 in size.[11–13] Despite their moderate success at addressing small-to-medium cartilage defects, these invasive treatments are only typically considered once significant cartilage surface erosion has occurred. Therefore, minimally invasive treatments that reduce or prevent cartilage erosion are of interest as early treatments.

One such approach is the use of injectable SF supplements. In an effort to delay or obviate surgical intervention, HA solutions are injected intra-articularly (i.e., viscosupplementation) to lubricate and cushion cartilage[14, 15]. Yet, there is significant controversy surrounding the use of viscosupplements (e.g., Synvisc, Orthovisc, Hyalgan, and Supartz) due to their limited clinical efficacy, susceptibility to hyaluronidase enzymatic degradation, and short residence time ($t_{1/2}$ = 1–3 days) in the synovial joint[16, 17]. Moreover, such viscosupplements have demonstrated chondroprotection only in ex vivo and limited small animal models.[18, 19] Hence, there is a need for new treatments that reduce cartilage friction and wear to provide chondroprotection.

We hypothesize that supplementing SF with a polymer that reduces the coefficient of friction (μ) will minimize cartilage wear, and ultimately delay or mitigate the need for more invasive OA treatments. Towards this goal, we are investigating high molecular weight polyelectrolytes as viscous biolubricants for cartilage. Several classes of cartilage protectants are under preclinical investigation[20] and include lubricin[21, 22] and lubricin mimics,[23–25] liposomes,[26] phospholipid coated silk microspheres,[27] and high molecular weight lubricious polymers,[28, 29] the latter of which can form viscous aqueous solutions due to polymer chain entanglement and/or crosslinking. From a tribological design perspective, a viscous lubricant creates a thin film between the cartilage surfaces to ensure that a pressurized fluid film more easily forms. This thin film reduces the prevalence of boundary lubrication, thus minimizing the harshest frictional loads. We recently reported the synthesis

of poly(7-oxanorbornene-2-carboxylate)[30] as an effective biolubricant in cartilage-on-cartilage torsional friction tests ex vivo[31] and in a rat model of osteoarthritis.[32] However, this polymer is highly anionic. To better match the osmolality of healthy SF (400 mOsm/kg, [33]), to reduce the overall negative charge of the polymer when dissolved in saline, and to examine the effect of altering the polymer architecture/composition, we synthesized a linear poly(7-oxanorbornene-2-carboxylate) of molecular weight and covalently conjugated pendent triethyleneglycol (TEG) chains to prepare a bottle-brush polymer lubricant of 2.8 MDa referred to as "2.8M TEG" after its molecular weight and TEG attachments (Figure 1). Herein, we report the synthesis of 2.8M TEG, its performance in three ex vivo long-duration friction tests compared to saline and bovine SF using intact and previously worn bovine cartilage, and initial biocompatibility studies after intra-articular injection in murine and canine models.

EXPERIMENTAL

Specimen Preparation and Study Design

Twenty-six mated, osteochondral plug pairs were cored from the femoral groove (12mm diameter) and patella (7mm) of seven freshly slaughtered, skeletally mature cows. All plugs were then frozen at −20 °C in 400 mOsm/kg saline containing GIBCO Anti-Anti stock solution (5x; Invitrogen, Grand Island, NY), ethylenediaminetetraacetic acid (5 mM; Sigma, St. Louis, MO), and benzamidine HCl (5 mM; Sigma B6506, St. Louis, MO). These same additives were included in all solutions that were exposed to the samples to prevent nonspecific cartilage degradation during the experiments as a result of the samples being left at room temperature or at 4 °C for extended periods. Prior to testing, all samples were thawed overnight at 4 °C. Twelve plug pairs were used to evaluate the frictional properties and wear prevention of saline, bovine synovial fluid (BSF), and 2.8M TEG during a long-duration torsional friction test (Study 1). In Study 2, three plug pairs were used to directly compare 2.8M TEG to BSF by testing the pairs in 2.8M TEG following testing in BSF. Finally, eleven plug pairs were used to evaluate the ability of BSF, Synvisc, and 2.8M TEG to improve the frictional properties of previously worn cartilage (Study 3). Group bias was controlled by using neighboring plug pairs from the same knees for each lubricant group.

Contrast Agent (CA4+) and 2.8M TEG Preparation

The computed tomography (CT) contrast agent CA4+ was synthesized as previously reported[34]. The contrast agent solution was prepared by dissolving the dry compound in deionized water, balancing the pH to 7.4 using NaOH, and adjusting the osmolality to 400 mOsm/kg using sodium chloride to match the in situ osmolality of articular cartilage (350–450 mOsm/kg[33]).

The linear 2 MDa poly(7-oxanorbornene-2-carboxylate) polymer was synthesized as previously reported[30, 31] (see Supporting Information, SI, for details), and triethyleneglycol (TEG) chains were added to a third of the available carboxylic acids using carbodiimide coupling in phosphate buffer at pH=6 to afford the 2.8 MDa polymer referred to as 2.8M TEG (Figure SI–1). The 2.8M TEG lubricant solution was then prepared by dissolving the

polymer in deionized water at 2 w/v%, balancing the pH to 7.4 using NaOH, and adjusting the osmolality to 400 mOsm/kg using sodium chloride.

Baseline Surface Photographing and microCT Imaging

For all studies, the congruent cartilage surfaces of the osteochondral plugs were photographed (1x and 2.25x magnification) using a microscope camera (PL-B681CU, PixeLINK, Ottawa, ON) to ensure all plugs had comparably smooth cartilage surfaces prior to friction testing. For Study 1, the plugs were then immersed in the CA4+ contrast agent at 12 mgI/mL (patella: 1 mL, groove: 3 mL) for 24 h at room temperature. The samples were then imaged on a microCT scanner (µCT40, Scanco Medical AG, Brüttisellen, Switzerland), and the microCT data were post-processed in Analyze™ (Analyze Direct, Overland Park, KS) using our previously developed protocols[35] (see SI). The thicknesses of the segmented cartilage object maps were then measured at 5 points across the width of the tissue for all coronal slices. Following contrast-enhanced CT (CECT) imaging, each sample was washed in excess saline for 24 hr to desorb the contrast agent. Since sample surface roughness was not examined in Studies 2 & 3, the cartilage thicknesses for these studies were measured using the same procedure as for Study 1, except the thicknesses were obtained from non-contrast-enhanced microCT scans.

Baseline Coefficient of Friction (µ) Testing

The baseline frictional properties of all plug pairs were evaluated at room temperature using saline as the test solution and the same mechanical testing procedure[36] (see SI). Briefly, each plug pair was compressed 18% (Enduratec 3230, BOSE, Eden Prairie, MN), relaxed for 70 min (stress equilibrium), and then the femoral groove plug was rotated against the patellar plug for 720° at 5°/sec[36]. Static and dynamic coefficients of friction (μ_{static}, μ_{static_eq}, and $\mu_{kinetic}$) were calculated (see SI), and all samples were then allowed to recover in saline overnight.

Long-Duration Friction Testing

The plug pairs in Study 1 were immersed in either the 2.8M TEG biolubricant (2 w/v%), BSF, or saline (n=4 plug pairs each; all plugs from the same animal) for 4 h at room temperature prior to long-duration testing as described previously (see SI for details).[32] Briefly, plugs from the femoral groove were rotated against the plugs from the patella at 360°/sec (22 mm/sec) for 10,080 rotations, while under 0.78 MPa compressive stress at room temperature (see SI). Every 160 rotations, there was a "lift-off" to allow lubricant re-introduction between the surfaces. The average µ (μ_{mean}) was calculated for each of the sixty-three 160-rotation periods between lift-offs. For Study 1 only, the rotations were paused every hour for extraction of a 100-µL lubricant aliquot for later analysis. After each test, the samples were allowed to recover for >16 h in saline.

For Study 2, three plug pairs were subjected to the same long-duration friction test on four sequential days. Using BSF as the lubricant, the first test was for preconditioning the samples. The lubricants for the subsequent tests (Tests 1–3) were BSF, 2.8M TEG (2 w/v%), and BSF, respectively.

For Study 3, all the plug pairs were subjected to the long-duration testing once per day on three sequential days: the first two days ("wear tests," lubricated by saline) were intended to progressively "wear" the cartilage, and the third day ("lubricant test") was to evaluate the efficacy of each lubricant (either BSF (n=4), Synvisc (n=3), or 2.8M TEG (2 w/v%) (n=4)) on "worn" cartilage.

Analysis of Wear

For all studies, the cartilage surface roughness of each plug was evaluated by photography as well as CECT as described previously[32] (see SI). Briefly, after the long-duration testing, circular grooves (rings) developed on some of the samples, indicating surface wear.[32] Two observers, blinded to the experimental groups, counted the number of rings in the surface photographs for the samples from Study 1 to evaluate the ability of the different lubricants to prevent cartilage surface wear. The average number of rings and standard deviation for each lubricant group was then computed.

Additionally, five sequential coronal and five sequential sagittal CECT images pre- and post-long-duration testing were evaluated to measure the change in each plug's cartilage surface roughness (see SI)[32, 37].

The mass of GAG in the lubricant solutions from Study 1 was measured using the 1,9-dimethylmethylene blue (DMMB) colorimetric assay[38] (see SI).

In Vivo Studies

Both preliminary in vivo studies were performed by Bolder BioPATH (Boulder, CO), approved by the IACUC at Bolder BioPATH, and were conducted in accordance with the recommendations in the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. For the first in vivo study (Study 4), male Lewis rats were injected in the right knee with 2 w/v% 2.8M TEG (N=4) or saline (N=4) and observed for any changes (left knee served as un-injected control). On day 3, animals were evaluated semiqualitatively for effect on gait and gait deficiency (no loads measured; see SI and Figure SI–2) and then euthanized on day 5. In Study 5, ten female beagle dogs were injected with 2 w/v% 2.8M TEG (N=5) or saline (N=5) into one knee joint. On day 4, animals were euthanized, and the synovium was sectioned and stained with H&E (See Table SI–1). All sections were examined by a board-certified veterinary pathologist for signs of inflammation (Dr. Alison Bendele).

Statistics

One-way ANOVA with Tukey post-hoc tests was used to evaluate differences between: 1) baseline μ for all lubricant groups within each study; 2) μ_{mean} for each lubricant group in Study 1; 3) number of "wear rings" on apposing cartilage surfaces for each lubricant group in Study 1; 4) percent increase in surface roughness after long-duration testing for each lubricant in Study 1; 5) mass of GAG in lubricant solutions from Study 1; 6) differences in μ_{mean} for Test 2 and Test 3 each normalized to Test 1 in Study 2; and, 7) the percent change in μ_{mean} for the "lubricant test" on day 3 relative to the "wear test" on day 2 for all time points and lubricants in Study 3. Significance level was set as two-tailed $p < 0.05$.

RESULTS

Synthesis

The poly(7-oxanorbornene-2-carboxylate) polymer was synthesized with a M_n of 2 MDa g/mol (PDI=1.2), and the TEG chains were successfully added to 30% of the polymer's carboxylate groups to give the 2.8 MDa polymer (2.8M TEG). Reaction yields for the two steps ranged between 80–95% (n=3), and the final 2.8M TEG (Figures 1 and SI–1) polymer was isolated as a white solid, similar in texture to HA (Figure 1c). The 2.8M TEG was completely soluble in water at 2 w/v%, and administered through a small 26G needle (Figure 1d). The viscosity of the 2.8M TEG 2 w/v% solution is ≈1.5 Pa·s at a shear rate of 1 Hz. For comparison, the viscosity of Synvisc is ≈1000 Pa·s, and does not readily pass through a 26G needle.

Long-Duration Friction Properties (Study 1)

There were no significant differences in the initial baseline coefficient of friction among the plug pairs assigned to saline, BSF or 2.8M TEG (2 w/v%) test groups for Study 1. The saline and BSF data reported herein were also used for comparison with the non-TEGylated predecessor to this lubricant[32] using plug pairs from the same bovine knees as this study to ensure cartilage plug consistency. Long-duration friction testing of plug pairs lubricated with 2.8M TEG exhibited the lowest μ_{mean} values, being statistically significantly lower for all time points after 5280 sec compared to plugs lubricated with saline, which had the greatest μ_{mean} results (Figure 2). The non-TEGylated polymer (2.4 MDa)[32] exhibited a lower μ_{mean} value of 0.016 lower compared to 0.027 for 2M TEG. The samples lubricated with BSF exhibited μ_{mean} values greater than those of 2.8M TEG and less than those of saline, being significantly lower than saline for 8 time points after 6240 sec of testing.

Wear Properties (Study 1)

Cartilage plug pairs lubricated with 2.8M TEG (2 w/v%) had fewer circular wear grooves on their surface compared to samples lubricated in saline but not BSF (Figure 3 and insert). Additionally, the samples lubricated with 2.8M TEG had noticeably less rough surfaces following long-duration friction testing than those tested in saline and BSF (Figure 4a). Comparing each femoral groove plug's post-testing surface roughness to its baseline value, the percent change in roughness after friction testing was significantly lower for samples tested in 2.8M TEG compared to samples lubricated with saline (Figure 4b). The average mass of GAG (a known wear debris product[39, 40]) in the 2.8M TEG test solutions was undetectable (<3 μg), while the mass of GAG found in the saline wear solutions was substantial at 121.1±33.1 μg (mean ± SD).

Directly Comparing 2.8M TEG to BSF (Study 2)

For Study 2, the overall mean coefficient of friction of Test2 (2.8M TEG; 2 w/v%) reduced by 20.9 ± 10.5% (mean ± SD) relative to that of Test1 (BSF), while the mean coefficient of friction of Test3 (BSF) returned to approximately the same value as that in Test1, being 2.0 ± 7.1% greater relative to Test1 (Figure 5), thereby demonstrating the reversibility of 2.8M

TEG's lubricating effect. Further, the normalized Test2 mean µ value was significantly less than the normalized Test3 µ value ($p < 0.05$).

Effectiveness of 2.8M TEG on Previously Worn Cartilage Surfaces (Study 3)

For Study 3, there were no significant differences in baseline µ among the three paired-plug groups assigned to BSF, Synvisc or 2.8M TEG (2 w/v%). The percent reduction in μ_{mean} for the day 3 "lubricant test" relative to the day 2 "wear test" was the greatest for 2.8M TEG, followed by Synvisc, then BSF (Figure 6), being significantly greater for 2.8M TEG than for BSF for all time points except 800, 6080, 6240, 6400, 6560, 6720, 6880, 7040 and 8480 sec ($p < 0.05$).

Rat and Dog In Vivo Studies

Rats injected intra-articularly with 2 w/v% 2.8M TEG gained approximately 7–8 grams of body weight over the course of the study and had no observed swelling, inflammation, or clinical abnormalities. The results were similar to animals injected with saline (N=4 per group). The rats treated with 2.8M TEG or saline had gait scores of 0 on day 3 after injection (see SI), with mean gait deficiency percentages of 3% for the 2.8M TEG treated animals compared to the saline treated animals. Dog knees injected with saline exhibited normal synovia, with one animal having minimal papillary proliferation (Figure 7a) (N=5 per group). Knees injected with 2 w/v% 2.8M TEG were comparable to saline-injected knees with two animals having minimal papillary proliferation following an established grading system (See SI; Table SI–1; Figure 7b).

DISCUSSION

To improve upon the shortcomings of current hyaluronic acid viscosupplements, we report a novel, synthetic bottle-brush polyelectrolyte based on poly(7-oxanorbornene-2-carboxylate) possessing TEG sidechains (2.8M TEG) and its efficacy as a cartilage lubricant (Figure 1). From a biomaterials and chemistry perspective, a polyelectrolyte that: 1) possesses an overall fixed negative charge will remain at the cartilage surface due to electrostatic repulsion from the internal cartilage negative fixed charge; 2) incorporates ethylene glycol units within the polymeric structure will enable a highly hydrated state; 3) adopts a bottle-brush polymer architecture reminiscent of lubricin to lubricate the surface; and, 4) possesses a molecular weight greater than 1 MDa to increase its joint space residence time by retarding its diffusion through the synovial membrane. Assuming a 2.8M molecular weight (16,495 repeat units) and TEGylation of 30% of the polymer repeat units, the polymer is highly charged with more than 10,000 formal negative charges. A 2 w/v% aqueous solution of the polymer is lubricious to the touch. Figure 2 shows the significant reduction in coefficient of friction (μ_{mean}) for plug pairs lubricated with 2.8M TEG (2 w/v%) compared to samples tested in saline and BSF. The observed plateau formation in the μ_{mean} curves indicates the cartilage interstitial fluid pressure has subsided (corroborated by a plateau formation at the same time in each group's corresponding creep curves). Such a plateau indicates a transition from predominantly hydrodynamic/elastohydrodynamic lubrication during the beginning portion of the test to predominantly either viscous lubrication (for a high-viscosity lubricant, e.g., 2.8M TEG) or boundary lubrication (for a low-viscosity lubricant) during the remainder

of the test[41]. The equilibrium values of μ_{mean} (defined as the mean of μ_{mean} in the fully plateaued region from 8000 to 10080 sec) for saline, BSF, and 2.8M TEG are 0.0626 ± 0.0015 (mean ± SD), 0.0448 ± 0.0016, and 0.0316 ± 0.0005, respectively. Several reports describe long-term coefficients of friction experiments using a cartilage-on-cartilage setup, [39, 42–44] and the most common configuration is linearly reciprocating sliding. However, our predecessor lubricant was evaluated using a torsional configuration, so the same setup was used to evaluate this lubricant for ease of comparison. Although it is difficult to compare μ values directly between torsional and linearly oscillating configurations, our results with saline are within an order of magnitude of these prior reports. For example, the lubricin mimic[23, 25, 45] lubricants afford $\mu_{kinetic}$ values ranging 0.1–0.3 on cartilage, while the liposomes[26] exhibit $\mu_{kinetic}$ values ranging 0.02–0.1 and phospholipid coated silk microspheres range 0.02–0.06.[27] A high molecular weight lubricious polymer with poly(2-methyl-2-oxazoline) sidechains gives $\mu_{kinetic}$ values spanning 0.02–0.1.[28] The 2.8M TEG biolubricant provides superior lubrication even after the interstitial fluid pressure has subsided, indicating the viscous lubricant is superior to healthy BSF and saline under these loading conditions.

Fitting an exponential of the form $\mu_{mean}=a*e^{-b*time}+c$ (MATLAB 2011a, MATLAB, Natick, MA) to the curves, the tau values (1/b), representing the time at which 63.2% of the plateau μ_{mean} value is reached[46, 47], are 4527 ± 2321 sec (mean ± SD), 3253 ± 4112, and 1070 ± 281 for saline, BSF, and 2.8M TEG, respectively. Although the samples were harvested from neighboring locations, the differences in μ curves and tau values for each lubricant are rationalized based on Stribeck principles. Since 2.8M TEG is substantially more viscous than saline, and the mechanical testing parameters are the same for both solutions, samples tested in the 2.8M TEG maintain mixed-mode lubrication as their interstitial-fluid pressure subsides, while samples tested in saline transition to boundary lubrication. During mixed-mode lubrication, the thin film between the cartilage surfaces reduces friction below that of boundary lubrication, hence the μ values for 2.8M TEG plateau sooner and lower than those of saline.

The presence of the polyelectrolyte biolubricant 2.8M TEG also affords significantly less cartilage surface wear than saline. The circular grooves that formed on the cartilage surfaces during testing (Figure 3) likely developed as thicker regions of the cartilage tissues shear against each other during the rotations. We hypothesize that when testing in saline, after the interstitial fluid pressure has subsided, the macroscopically smooth cartilage wears at these high-stress regions as any remaining hyaluronic acid (HA) or lubricin molecules on the cartilage surfaces are forced out of the interface. However, when testing in BSF, HA and lubricin macromolecules can re-deposit between the cartilage surfaces during liftoffs, thus preventing as much direct matrix-matrix contact, which results in less surface wear. When the 2.8M TEG is used, these large polymers reside between the cartilage surfaces, affording fewer wear rings (Figure 3). This hypothesis of a lubricating polymer's resistance to being squeezed away from an articulating surface is supported by other investigations of polymer morphology under tribological load, with polymer chains flattening yet not dissipating from the interface.[48, 49] To further analyze the cartilage surface roughness, we extended a technique originally developed for analyzing Safranin-O stained histology slices[37] to our cartilage CECT data. The samples tested in saline experience a ~7.22x increase in surface

roughness compared to baseline, while samples tested in BSF and 2.8M TEG undergo a 2.62x and 1.05x increase, respectively (Figure 4). These results agree with the mass of GAG released into the lubricant solutions during testing. More GAG is lost from the cartilage samples tested in saline (121.1 ± 33.1 μg (mean ± SD; consistent with literature reports[39, 40]) than from those tested in 2.8M TEG (<3 μg). Hence, use of the 2.8M TEG biolubricant minimizes cartilage surface wear.

To directly compare the performance of BSF and 2.8M TEG (2 w/v%), the same cartilage samples are used with both lubricants (BSF, wash, 2.8M TEG, wash, BSF) to determine if: 1) 2.8M TEG reduces μ below that of BSF; and, 2) 2.8M TEG is washed from the cartilage surfaces after testing such that when the samples are tested in BSF again their μ values return to the original BSF μ values. Pilot studies using BSF confirm that after the first pre-conditioning long-duration test, the next three tests afford comparable μ_{mean} values (data not shown). Treatment of the cartilage surfaces with 2.8M TEG reduces μ by 20.9 ± 10.5% (mean ± SD) from that of BSF (Figure 5). The ability of the 2M TEG to reduce the μ value is not hindered by a plug coated or previously treated with BSF. Further, the percent change between the two BSF tests is 2.0 ± 7.1%, indicating that 2.8M TEG is likely washed from the cartilage surfaces and BSF is the active lubricant in the final test as the μ value returned to its original level. The 2.8M TEG is rinsed away, because it does not bind to or alter the cartilage surface. Its negative charge electrostatically repels it from the fixed negative charge in cartilage. Hence, the polyelectrolyte acts as a viscous lubricant and forms a layer interposed between the two cartilage surfaces during testing. The 2.8M TEG biolubricant will likely be slow to diffuse from an intact joint, as its molecular weight is greater than the largest pores of the joint capsule (~750 kDa molecular weight cut-off).[50]

Finally, the performance of BSF, Synvisc, and 2.8M TEG (2 w/v%) are compared using previously worn cartilage. Since samples tested in saline during the long-duration test become substantially rougher than at baseline, the samples in this study are intentionally worn via two long-duration tests using saline to impart surface roughness prior to a third test with one of the lubricant groups. Despite the increased roughness, 2.8M TEG still reduces the coefficient of friction by ~60%, compared to ~35% for Synvisc and ~15% for BSF, being significantly different than BSF for most of the time points (Figure 6). The 2.8M TEG is the first reported lubricant, to our knowledge, to reduce friction of roughened ex vivo cartilage surfaces, while HA-based viscosupplements are relatively less effective at lubricating degraded cartilage. For example, the coefficient of friction of HA treated samples increases by approximately 25% after cartilage is worn,[18] where as the coefficient of friction of 2.8M TEG treated samples decreases by approximately 20% after the cartilage was worn.

The 2.8M TEG (2 w/v%) polymer also exhibits positive preliminary safety in vivo with respect to gait and inflammation. There is no change in gait score (compared to untreated animals) or signs of abnormalities after intra-articular administration in rats. Likewise, intra-articular injection of 2.8M TEG affords effects similar to the saline control in dog knees (Figure 7). Although extended-duration timepoints were not investigated, the preliminary data from this pilot study indicate minimal acute safety concerns, thus providing motivation for further *in vivo* safety and efficacy evaluation.

CONCLUSIONS

In osteoarthritis, both cartilage and synovial fluid progressively deteriorate, which reduces joint lubrication. In this study, the 2.8M TEG polyelectrolyte reduces cartilage wear by lowering the coefficient of friction during long-duration testing and is superior to BSF and Synvisc for lubrication of ex vivo worn cartilage. Future studies using healthy and osteoarthritic human cartilage are planned to validate our findings and further challenge this biolubricant as a potential treatment for early-stage OA. Nevertheless, the present studies demonstrate 2.8M TEG's enhanced lubrication for both healthy and worn cartilage in more abrasive loading conditions than those encountered in vivo, as articulating joint surfaces move at slower speeds than those tested herein and are more continuously re-coated with lubricant than the present samples were. Treatment with the 2.8M TEG affords minimal acute in vivo synovitis similar to treatment with saline warranting further in vivo safety and efficacy studies. In summary, a long-lasting, intra-articularly injected biolubricant, such as 2.8M TEG, potentially fulfills the unmet need for a minimally invasive treatment option for patients with early-stage cartilage wear who wish to decelerate their OA and delay a joint replacement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

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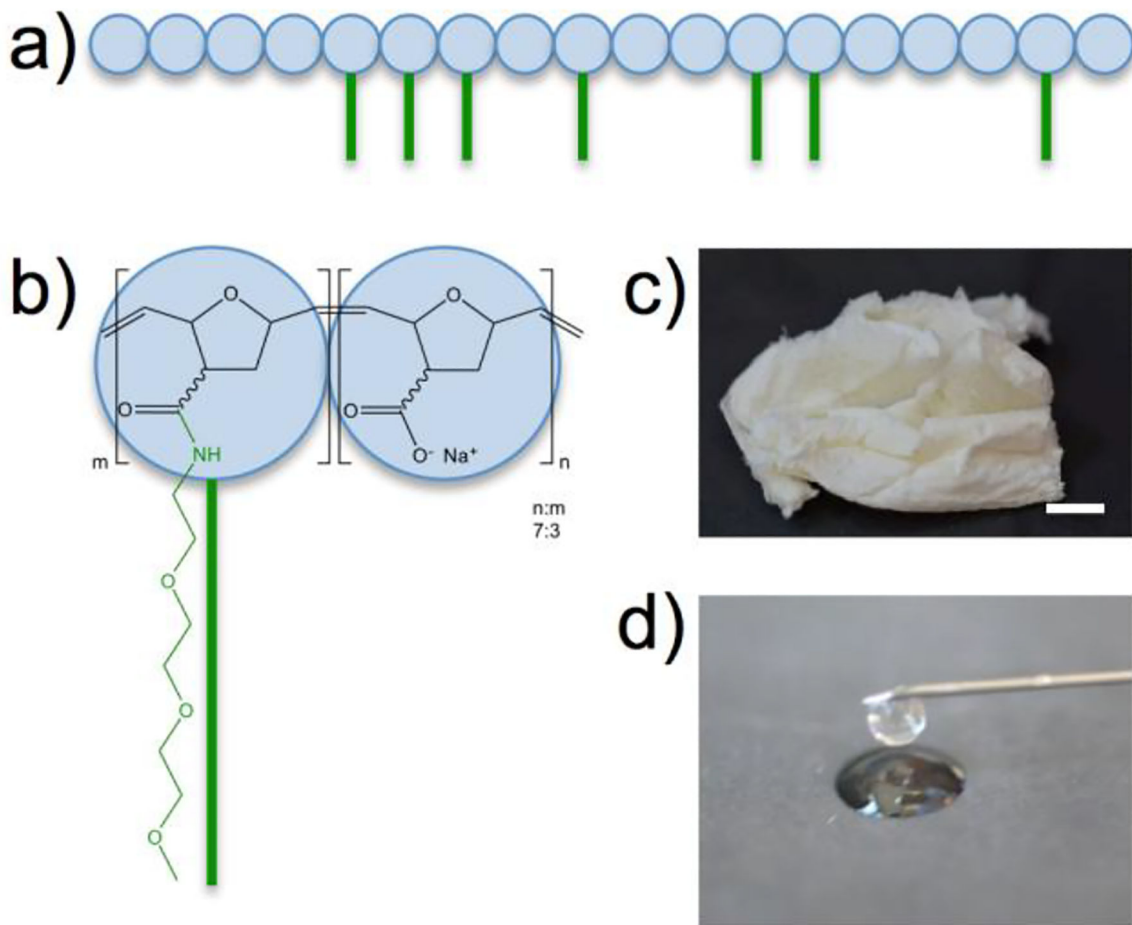
Figure 1.
a) Graphical representation of the bottle brush 2.8M TEG lubricant. **b**) Chemical structure of the 2.8M TEG polymer. **c**) Photograph of the isolated 2.8M TEG polymer after synthesis. Scale bare = 1 cm **d**) Photograph of an aqueous solution of the 2w/v% 2.8M TEG ejecting from a 26G needle.
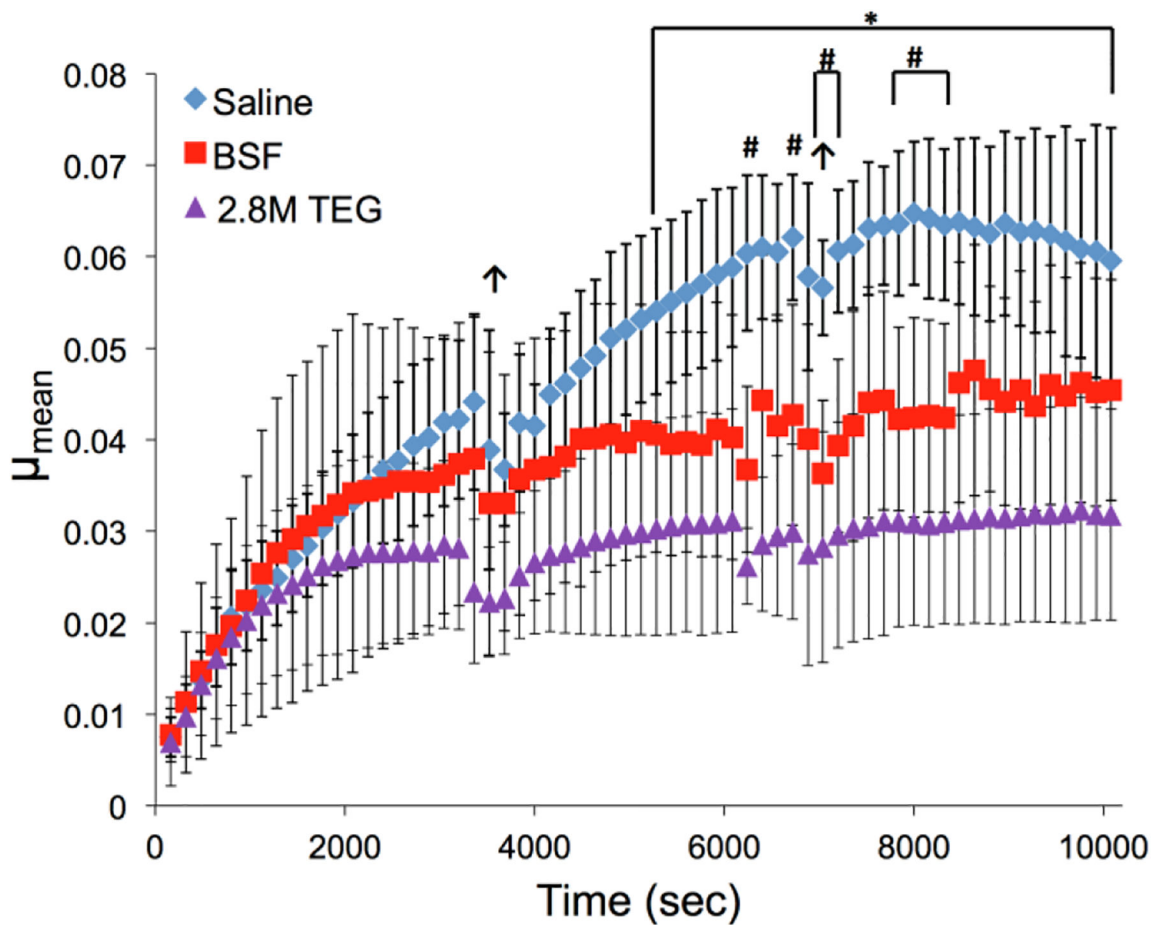
Figure 2.
Coefficients of friction (μ_{mean}) for bovine cartilage plug pairs (patella against femoral groove) tested in three lubricants (n=4 each) during the long-duration torsional friction regimen (>10,000 rotations) of Study 1. Error bars indicate standard deviation. *2.8M TEG vs Saline (p<0.05), #BSF vs Saline (p<0.05), ↑ indicates pause for lubricant extraction (plugs held apart for ~30 sec). Saline and BSF data reproduced with permission from ref 32. Copyright 2018 Elsevier.
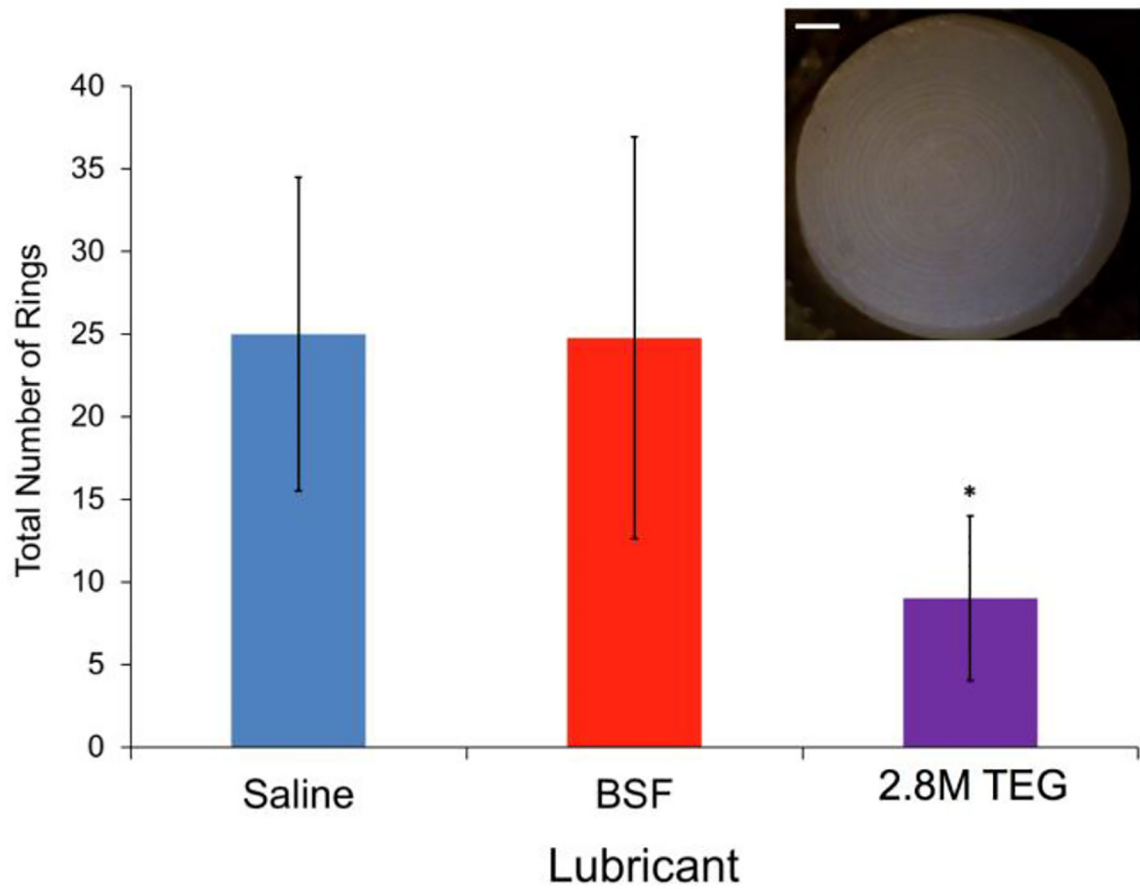
Figure 3.
Total number of rings on cartilage plug surfaces from each pair tested (example photo shown in inset) following long-duration torsional friction testing in three lubricants (n=4 each) in Study 1. Error bars indicate standard deviation. *2.8M TEG vs saline (p<0.05). Scale bar is 1 mm.

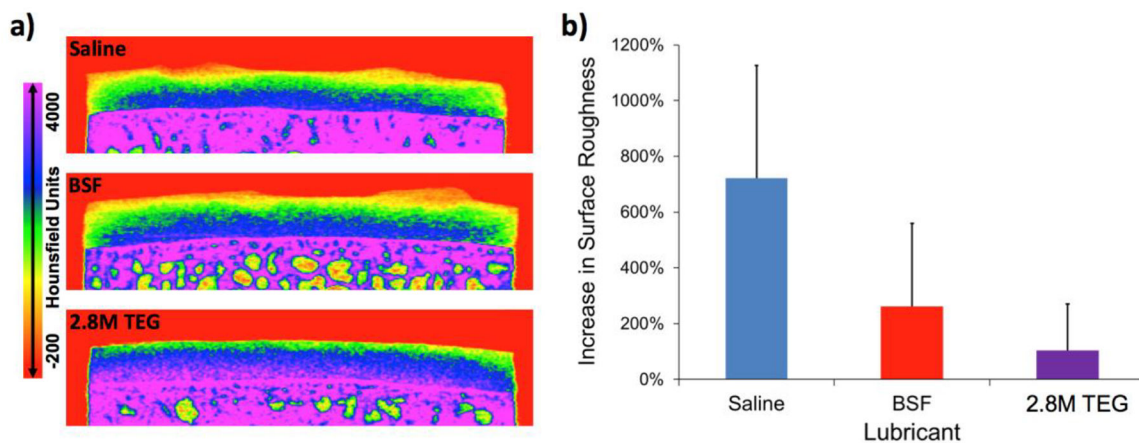**Figure 4.**
a) Representative CECT color maps of femoral groove plugs subjected to long-duration torsional friction testing in Study 1 using saline, BSF, and 2.8M TEG. **b)** Percent increase in cartilage surface roughness following long-duration torsional friction testing in three lubricants (n=4 each) during Study 1. 2.8M TEG vs Saline (p<0.05). Error bars indicate standard deviation.
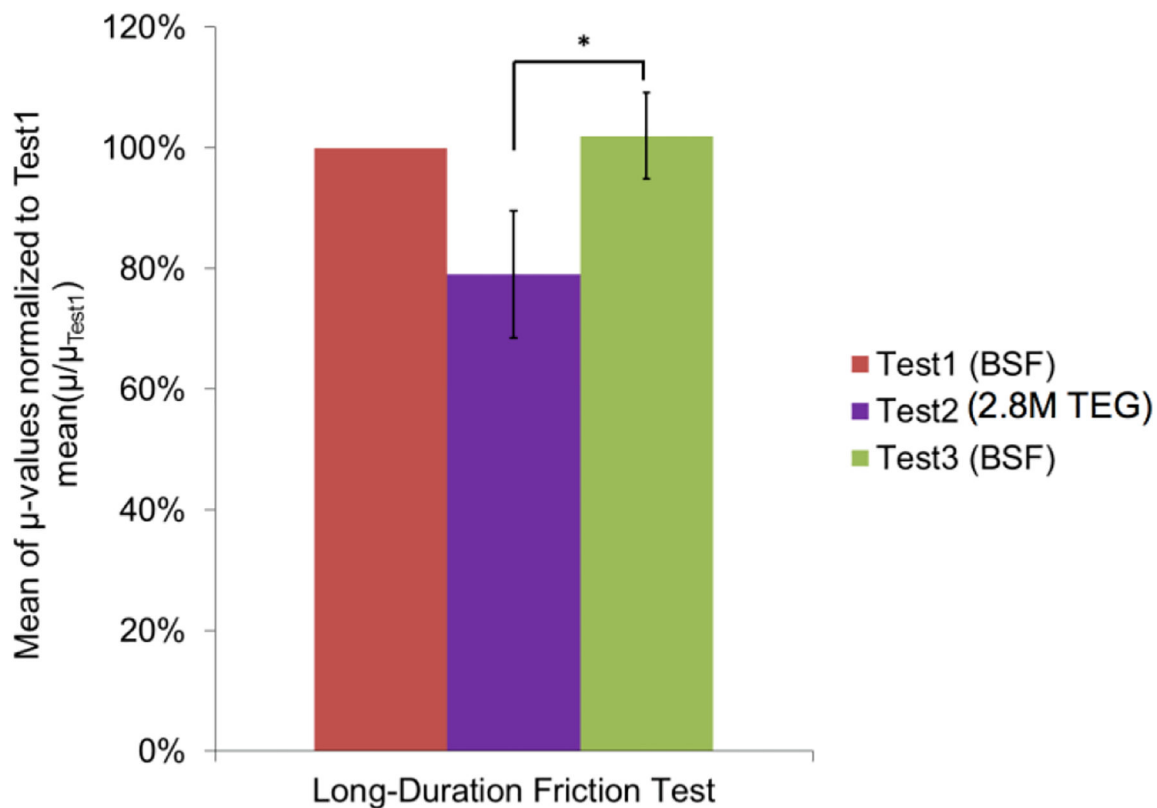
Figure 5.
Mean of each long-duration friction test's μ-values normalized to the μ-values of Test1 from Study 2 (n=3). The mean coefficient of friction of Test2 (2.8M TEG) reduced by 20.9 ± 10.5% (mean ± SD) relative to that of Test1 (BSF), while the mean coefficient of friction of Test3 (BSF) increased by 2.0 ± 7.1% relative to Test1. Error bars indicate standard deviation. *The normalized Test2 mean μ-value was significantly less than the normalized Test3 μ-value ($p<0.05$).
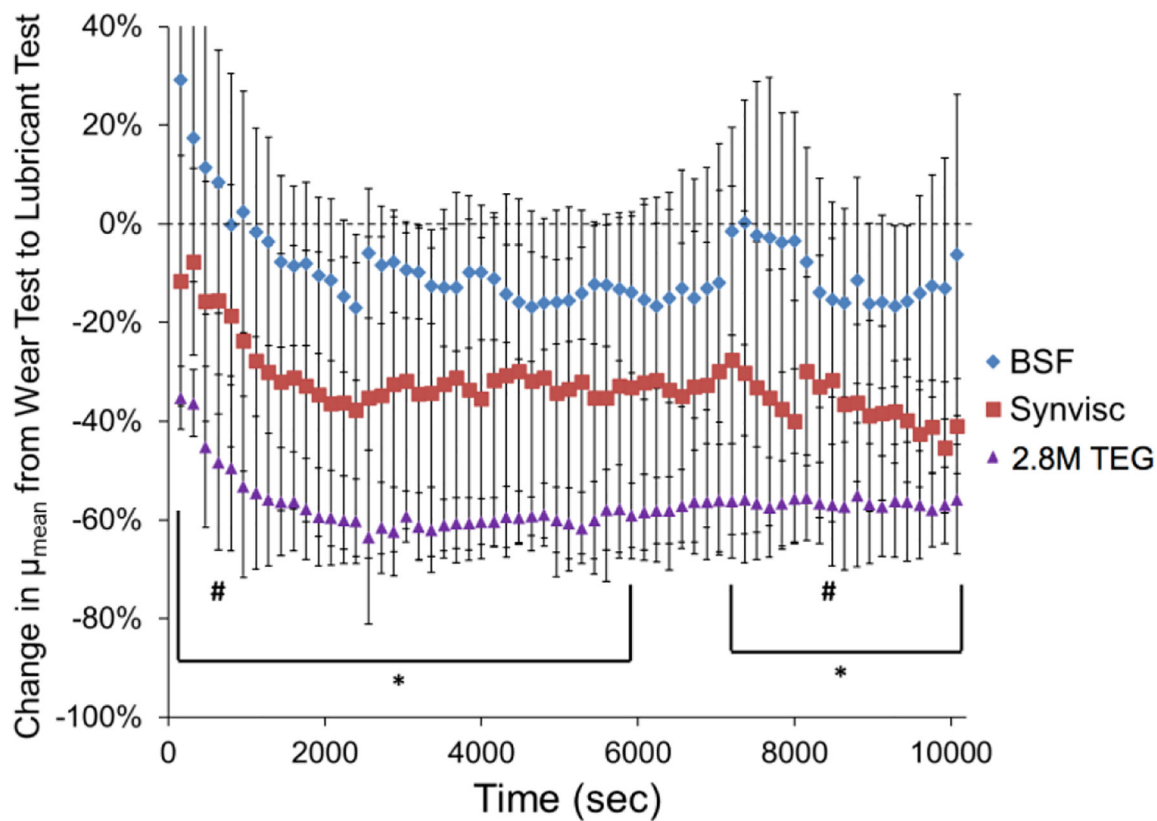
Figure 6.
Percent change in μ_{mean} from the day 2 "wear test" to the day 3 "lubricant test" in BSF
(n=4), Synvisc (n=3) and 2.8M TEG (n=4) during Study 3. Error bars indicate standard
deviation. The percent change in μ_{mean} values for 2.8M TEG were significantly lower than
those for BSF for all time points except 800, 6080, 6240, 6400, 6560, 6720, 6880, 7040 and
8480 sec. *2.8M TEG vs BSF (p<0.05), except for the two time points indicated with # (800
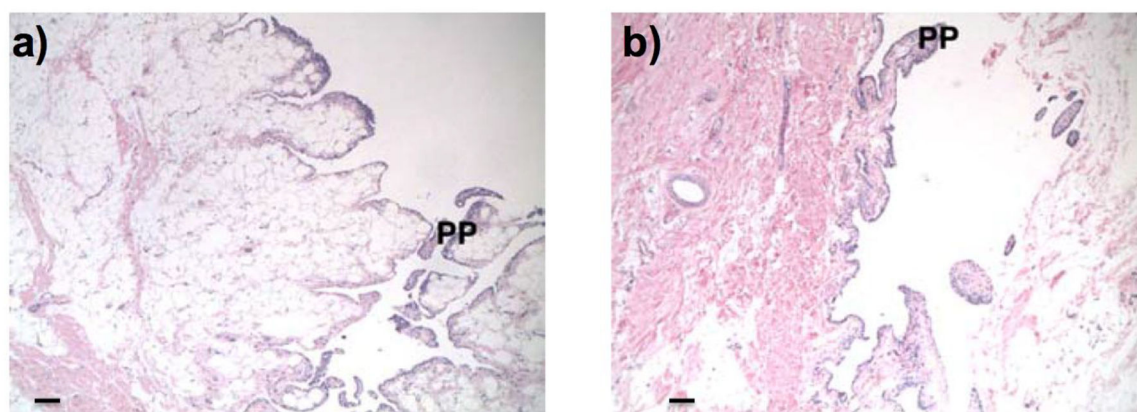and 8480 sec).

Figure 7.
Representative H&E histological sections (50x) of synovium from beagle knees injected with **a)** saline or **b)** 2 w/v% 2.8M TEG. Scale bar = 100 μm. Both knees experienced minimal papillary proliferation (PP).