Synovial Fluid Reduces Immature Bovine Cartilage Wear By Fatigue Failure Compared To Saline

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INTRODUCTION: Osteoarthritis (OA) is a disease of the joint characterized by the wear and degeneration of the articular surface of cartilage. It has been previously shown in immature bovine cartilage that the primary mechanism of wear is through fatigue failure and subsequent delamination of the superficial zone (SZ) from the middle zone (MZ) of the tissue layers [1, 2]. Synovial fluid (SF) has been shown to have a beneficial effect in reducing the rate of wear in the mouse model [3, 4]. However, this reduction in wear was previously attributed to the role of boundary lubricants in SF that reduce cartilage frictional forces. Although cartilage wear is often assumed to result from frictional forces during sliding, recent results have demonstrated that cartilage fatigue failure is due to reciprocal compressive stresses and not frictional stresses [5]. In this study we hypothesize that SF reduces cartilage wear by enhancing the resistance of the cartilage extracellular matrix (ECM) to fatigue failure. We test this hypothesis by examining reciprocal compressive sliding of immature bovine cartilage using SF versus phosphate buffered saline (PBS).

METHODS: Full thickness articular cartilage tibial plateaus were harvested from 8 immature bovine knees (4 left, 4 right) and microtomed from the deep zone (DZ) to a thickness of 1.39±0.07 mm. Samples were paired from the medial and lateral plateaus of each joint and assigned to the PBS group (n = 8) and the SF group (n = 8). The plateaus were cut to form 10 mm × 30 mm strips and mounted to the center of a petri dish using Loctite. Prior to testing, samples were photographed and then scanned using a laser scanner (Keyence Profilometer, LJ-V7080) to create a dense point cloud (50,000 points/cm^2) for surface deviation calculation (R_q). The sample pairs were placed into two custom friction testing devices with a reciprocating stage that collects position and load data as described previously [6]. Both samples were oriented with the long axis of the strip along the sliding x-direction, and brought into contact with a hemispherical glass lens (∅ 25 mm) applying 4.45 N of static compressive load. Contact area was assessed before testing using pressure sensitive contact film (FujiFilm). On one device, 15 mL of PBS was added to hydrate the sample in the PBS group. In the other device, 15 mL of mature bovine SF was added to hydrate the sample in the SF group. Both the PBS and the SF were supplemented with inhibitors of tissue degradation (0.04% Proclin, 0.1% EDTA). Reciprocal sliding was then applied at a speed of 1 mm/s across a distance of U_x = ±4 mm for all samples. After 24 hours (5,400 cycles), both samples were taken off the tester and photographed to assess damage. If significant visual damage was observed, the sample was taken off the tester and the friction test was terminated after the first 5,400 cycles. If no visual damage was observed, the sample was placed back onto the tester with fresh bath solution (either PBS or SF) and the test was continued for an additional 48 hours resulting in a total of 16,200 cycles. In either case, immediately after testing was terminated, contact area was again assessed, photographs were taken, and a scan to assess surface deviation was conducted. The surface deviation was calculated by taking the root mean square error of the point cloud data relative to a fitted plane for the tract length (8×8 mm). Subsurface damage was evaluated using polarized light microscopy of 120 μm sections along the axis of sliding. One-way analysis of variance (ANOVA) was performed on the contact area A, friction coefficient μ, and surface deviation R_q to compare the PBS and SF groups. Repeated measures ANOVA was performed on the samples before and after treatment for deviation measurements (ncss.com).

RESULTS: After 24 hours, all 8 PBS samples showed gross physical damage (Figure 1) and testing was terminated after 5,400 cycles. None of the SF samples showed gross visual damage after 24 hours and were thus allowed to run for 16,200 cycles (72 hours total). Only 2 out of the 8 SF samples showed gross physical damage after 72 hours of testing. Before testing, contact stresses between the PBS group (0.66±0.38 MPa) and the SF group (0.55±0.16 MPa) were not significantly different (p=0.46). After testing, the PBS group had a contact stress of 0.54±0.38MPa, not significantly different from the SF group, 0.50±0.14 MPa (p=0.76). The average friction coefficient for PBS (μ=0.028±0.011) was significantly higher (p<0.001) than the average friction coefficient of the SF group (μ=0.011±0.002). The average surface deviation before testing was not significantly different for the PBS and SF groups (p=0.185, R_q=0.049±0.015 mm for PBS, R_q=0.041±0.007 mm for SF). After testing, the difference became significant (p<0.001), with R_q=0.218±0.048 mm at 24 h in PBS being higher than R_q=0.057±0.024 mm in SF at 72 h. The PBS group showed a significant increase in deviation before and after testing (p<0.001), and the SF group showed no significant increase in deviation after testing (p=0.34). Analysis of PLM supported the indication of gross damage in all 8 samples in the PBS group and 2 samples in the SF group (Figure 2).

DISCUSSION: Our results demonstrate that SF reduces the incidence of fatigue failure in immature bovine tissue in comparison to PBS. After 24 h (5,400 cycles) of testing, all eight samples in the PBS group showed significant damage whereas none of the SF samples showed any gross visual damage. After 72 h (16,200 cycles), only three of eight samples tested in SF exhibited damage, thus validating the hypothesis that SF delays the onset of fatigue failure in immature bovine cartilage compared to PBS. While the friction coefficient in SF was statistically smaller than PBS in this study, both were very low, and we do not attribute the enhanced wear resistance to the reduced friction coefficient in SF, based on the experimental evidence presented in [5]. Instead, the results reported here suggest that molecular species present in SF, and absent from PBS, might diffuse into the SZ and impart a protective role to the collagenous ECM, increasing its resistance to fatigue failure. Fatigue failure is a classical wear phenomenon by which covalent bonds in the material progressively break over time in response to loading, until the remainder of intact bonds all break in a sudden failure event. Here, it appears that some molecular species in SF that bind to the ECM may enhance the strength of its covalent bonds [7]. Future investigations will examine fatigue failure of cartilage in testing configurations that specifically preclude friction.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding that fatigue failure at the interface of the SZ and MZ represents the earliest onset of mechanically-mediated degeneration of articular cartilage in OA may render this region a primary target for repair strategies in early OA.

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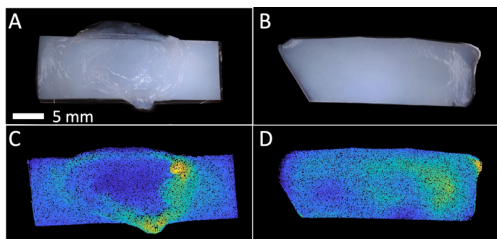
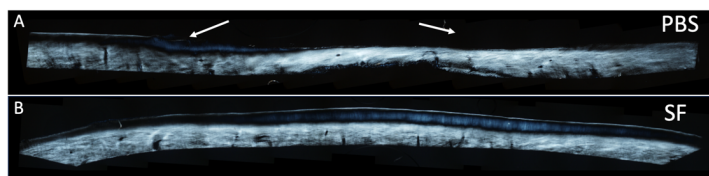Fig. 1: Photos and scans for a representative PBS (A,C) and SF (B, D) sample.

Fig. 2: PLM images for a representative PBS (A) and SF sample (B). Arrows indicate torn SZ.