Synovial Fluid Delays Delamination Wear in Immature Bovine Cartilage Under Pure Cyclical Compressive Loading

Haoyu Zhang[1], Serdar Kaltalioglu[1], Juliette Audric[2], Clark T. Hung[1], Gerard A. Ateshian[1]
[1]Columbia University, New York, NY, [2]École Polytechnique, Palaiseau, France
hz2831@columbia.edu

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INTRODUCTION: Articular cartilage, critical for load-bearing joints, is expected to offer a low-friction, wear-resistant surface capable of enduring millions of loading cycles over a lifetime. During daily activities, it often experiences cyclical compressive forces that can lead to fatigue failure [1], typically as delamination within the middle zone (MZ) [2]. Recent studies suggest that synovial fluid (SF) significantly delays cartilage fatigue failure under cyclical compressive loading, as produced by reciprocating sliding contact [1,3]. Peterson et al. first showed that SF induces less cartilage wear than phosphate-buffered saline (PBS) under a migrating contact area (MCA) configuration [1]. Building on this work, Sise et al. further investigated the role of SF and demonstrated that it reduces fatigue failure under cyclical compression with sliding in immature bovine cartilage [3]. While these novel findings highlight the importance of SF's protective role, our claim that the protective effect of SF lies not in reducing the friction coefficient of cartilage, but increasing its resistance to fatigue failure, remains novel and arguably controversial. Peterson et al. used MCA (cyclical compression + cyclical friction) and stationary contact area (SCA, static compression + cyclical friction) configurations to show that wear was driven by cyclical compression: MCA samples delaminated, while SCA samples—despite higher friction coefficient—did not [1]. However, both studies by Peterson et al. and Sise et al. paired compression with frictional forces, leaving the isolated role of cyclical compression arguably untested. Thus, direct evidence isolating SF's effect under pure cyclical compressive loading (without sliding) is lacking. In the present study, we addressed this gap using a customized, solenoid-based fatigue tester that applies cyclical compressive loading without concurrent sliding. Based on our prior findings [1,3], we hypothesize that SF delays the onset of delamination wear under pure cyclical compressive loading.

METHODS: Sample Preparation: Immature bovine knees (2–3 months old, n = 4) were obtained from a local abattoir and dissected to expose the femoral condyles. Articular surfaces were stained with India ink and rinsed with PBS to exclude samples with visible fibrillation or damage. Cylindrical cartilage plugs (∅ 12 mm) were harvested from both medial and lateral femoral condyles using biopsy punches. Samples were then sectioned from the deep zone to a final thickness of 1.75 ± 0.08 mm using a freezing-stage sledge microtome, retaining only articular cartilage. Each plug was mounted at the center of a ∅ 60 mm petri dish using minimal cyanoacrylate. Samples were stored in PBS supplemented with 0.04% isothiazolone-based biocide (ProClin 950, Millipore Sigma, #46878-U) and 0.1% protease inhibitor (0.5 M EDTA, Sigma-Aldrich, #03690) and kept frozen at –7°C until testing. Synovial fluid (100 mL) from mature bovine joints (Lampire Biologics, #8620853) was centrifuged at 3000 g for 20 minutes to remove impurities, supplemented with the same inhibitors as PBS, aliquoted, and stored at –20°C until use. Mechanical Testing: Cartilage plugs were tested using a custom-built, solenoid-based, load-controlled cyclical compression system. Paired samples from four knees (2 left, 2 right) were assigned to PBS (n = 4; 2 medial, 2 lateral) or SF (n = 4; 2 medial, 2 lateral) groups. Each plug was submerged in 15 mL of its respective medium (PBS or SF + inhibitors) and tested. Group assignments alternated between medial and lateral samples to control for age. A 53.4 ± 0.6 N compressive load was cyclically applied using a plano-convex glass lens (∅ 12.5 mm) at 2 Hz for up to 36,000 cycles. Photographs, contact area measurements, and surface topography scans were collected at baseline and after 12,000, 24,000, and 36,000 cycles to assess damage progression. Tests were terminated at 12,000 cycles if gross visual damage was observed; otherwise, they continued to 36,000 cycles over 5 hours.

RESULTS: Contact pressure (p_{avg} = 3.75 ± 0.53 MPa) and surface roughness (R_q = 0.029 ± 0.004 mm) prior to testing showed no significant differences between PBS and SF groups (p = 0.46; p = 0.85, respectively). After testing, all PBS samples exhibited gross visual damage (**Fig. 1D**) by 12,000 cycles of loading, thus testing was terminated. In contrast, none of the SF samples failed by 12,000 cycles, and all continued to 36,000 cycles without evidence of gross visual damage (**Fig. 1B**). Significant increases in surface roughness occurred in the PBS group (p = 0.0051), but not in the SF group (p = 0.68) (**Fig. 2**). Polarized light microscopy (PLM) confirmed delamination in the MZ layer, in PBS samples, with no detectable damage in SF samples (**Fig. 3**).

DISCUSSION: Cartilage plugs tested under cyclical compression in PBS, at a physiological level of contact pressure, all failed at 12,000 cycles of loading, whereas contralateral samples tested identically in SF did not fail even up to 36,000 cycles (**Fig. 1-3**). These findings strongly support the hypothesis of our study, providing direct evidence that SF's protective effect against fatigue failure by delamination does not arise from an improvement in boundary lubrication. This finding confirms a novel functional role of SF in mitigating compressive fatigue failure, as delamination could be attributed solely to cyclical compressive forces—unlike our prior cyclical sliding-based testing configurations [1,3]. The damage noted in this study appeared as delamination initiating in the MZ, located approximately 200 μm below the articular surface, identical to the delamination observed in our earlier frictional tests under MCA [1,3]. The strong protection imparted by SF became evident as early as 12,000 cycles (~100 minutes) after test initiation, strongly suggesting that low-molecular-weight SF constituents—capable of diffusing into the MZ within physiological residence times without tightly binding to the extracellular matrix (ECM) [4-6]—may be responsible for this protective effect. Likely candidates include soluble molecules such as bovine serum albumin (BSA, 66.5 kDa), γ-globulin (155–160 kDa), and surface-active phospholipids (SAPL, 700–800 Da) [7-9]. Based on their molar masses [10], diffusivities were estimated at 4.1–4.7 × 10^{-5} mm^2/s, corresponding to transport times of <11 minutes across 200 μm (<20 minutes with ECM hindrance [11]). Such rapid diffusion times support these solutes as plausible mediators of SF's ability to delay the onset of delamination under cyclic compressive loading, highlighting a potential direction for future investigations.

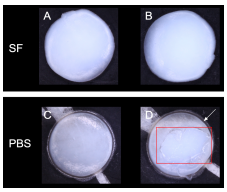

Fig. 1. Representative images before (**A**, **C**) and immediately after (**B**, **D**) testing. The region of delamination is outlined in red.

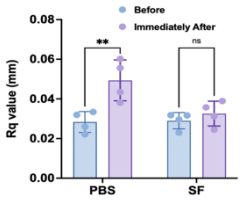

Fig. 2. Surface roughness (Rq, mm) before (blue) and immediately after (purple) testing. **Significance**: $p < 0.01$ (**).

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides incontrovertible evidence that SF protects cartilage from fatigue failure under cyclical compressive loading, independently of any putative effect it may provide toward the reduction of the friction coefficient between articular surfaces. This novel understanding of the role of healthy SF may have a profound impact on the early treatment of osteoarthritis.

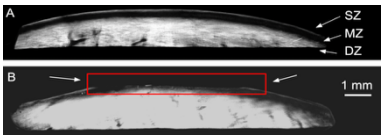

Fig. 3. Representative PLM images after testing: **(A)** Undamaged SF sample with intact superficial (SZ), middle (MZ), and deep (DZ) zones labeled; **(B)** Damaged PBS sample with the region of delamination highlighted by a red box.

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