

Fig. 2. Placebo-effect by pain category.

**64 CHONDROCYTE DEATH AND MITOCHONDRIAL DYSFUNCTION ARE MEDIATED BY CARTILAGE FRICTION AND SHEAR STRAIN**

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**Purpose:** Both injury and disease reduce synovial fluid's ability to lubricate, and recently inferior lubrication was linked to chondrocyte apoptosis. However, the specific mechanical and biological mechanisms that lead to this apoptosis remain unknown. Both cell death and mitochondrial (MT) dysfunction within articular chondrocytes are correlated with tissue after impact. Additionally, tissue strain during articulation is affected by both lubricant composition and tissue depth. The goals of this study were: 1) to measure the depth-dependent shear strains of cartilage for friction coefficients that span the range between injury and disease, and 2) to connect these results to cellular changes within articular cartilage including cell death and MT dysfunction. Collectively, these data will help to describe a link between inferior lubrication may and the progression of cartilage damage.

**Methods:** Cartilage was obtained from the femoral condyles of neonatal bovinds. Friction coefficients were measured against glass under 15% normal strain while lubricated by PBS or equine synovial fluid (ESF). Local shear strains during sliding were measured by tracking the displacements of photobleached lines with a confocal microscope. Also, samples were articulated under the same conditions for 30 cycles at 1 mm/s then allowed to reequilibrate in DMEM for 90 minutes. Slid samples and controls were stained with either calcein AM (live cells) and ethidium homodimer (dead cells) or MitoTracker Green (MTrG, all MT) and tetramethylrhodamine methyl ester (TMRM, functional MT). Percent cell death and MT dysfunction were calculated using ImageJ.

**Results:** The friction coefficient of cartilage lubricated by ESF was 70% lower than PBS (0.08 to 0.26, Fig 1A), and depth-dependent shear strain profiles scaled similarly (Fig 1BC). In the ESF lubricated cartilage, shear strains near the surface were under 3% and reached 1–2% in deeper tissue, but for the PBS group, shear strains were over 8% near the surface and under 5% in deeper tissue. In non-slid control tissue, minimal cell death was found (Fig 2CD). For ESF-lubricated cartilage, cell death was localized to the superficial 50 μm of tissue (Fig 2BD). However, for PBS-lubricated cartilage, dead cells were located over 100 μm from the articular surface (Fig 2AD). In contrast to cell death data, sliding in both PBS and ESF caused MT dysfunction in the superficial 100 μm of tissue. For control tissue, colocalization of MTrG (staining all MT) and TMRM (staining only polarized MT) occurred within functional chondrocytes near the surface (Fig 2EG). However for both ESF and PBS, colocalization of both MTrG and TMRM was rare within the superficial tissue (Fig 2E-G).

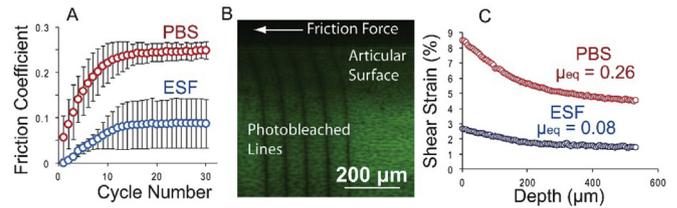


Figure 1. (A) Friction coefficient vs sliding speed (n = 4). (B) Local shear strains were tracked on a confocal microscope. (C) Local shear strains were high in PBS lubricated tissue compared to control.

**Conclusions:** This study connected cartilage friction coefficient with depth-dependent shear strains in cartilage to understand mechanical cues that lead to both cell death and MT dysfunction. We revealed significant cell death within the superficial 100 μm of cartilage lubricated by PBS where shear strains ranged from 6% to 8%. This region is known to have increased apoptosis in joints with high boundary friction. Conversely, minimal cell death was detected in the ESF-lubricated tissue, likely due to the lower shear strains (~2%). Interestingly, both ESF- and PBS-lubricated cartilage displayed mitochondrial dysfunction within the superficial 100 μm of tissue. While this result can be attributed to cell death in the PBS group, it is likely live cells in the ESF group that may be destined for MT-mediated apoptosis. Notably, although the deeper chondrocytes in the PBS group were exposed to high shear strains (~5%) there was little evidence of MT dysfunction, however, superficial cells in the ESF group experience lower shear strains (~3%) but displayed pronounced MT dysfunction. This phenomenon may indicate that the chondrocytes in the different regions of articular cartilage respond differently to mechanical signals regarding both necrosis and apoptosis, and that dysfunctional cells may be an important target for restabilization and ultimate prevention of PTOA.

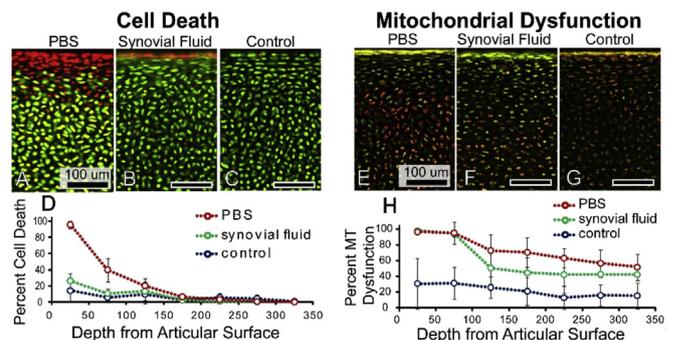


Figure 2. (A-C) Live/dead staining. (D) Percent cell death vs tissue depth. (E-G) MT (green) and active MT (red) staining. (H) Percent MT dysfunction vs tissue depth (n = 4).