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Short communication

Mapping the spatiotemporal evolution of solute transport in articular cartilage explants reveals how cartilage recovers fluid within the contact area during sliding

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ABSTRACT

The interstitial fluid within articular cartilage shields the matrix from mechanical stresses, reduces friction and wear, enables biochemical processes, and transports solutes into and out of the avascular extracellular matrix. The balanced competition between fluid exudation and recovery under load is thus critical to the mechanical and biological functions of the tissue. We recently discovered that sliding alone can induce rapid solute transport into buried cartilage contact areas via a phenomenon termed tribological rehydration. In this study, we use in situ confocal microscopy measurements to track the spatiotemporal propagation of a small neutral solute into the buried contact area to clarify the fluid mechanics underlying the tribological rehydration phenomenon. Sliding experiments were interrupted by periodic static loading to enable scanning of the entire contact area. Spatiotemporal patterns of solute transport combined with tribological data suggested pressure driven flow through the extracellular matrix from the contact periphery rather than into the surface via a fluid film. Interestingly, these testing interruptions also revealed dynamic, repeatable and history-independent fluid loss and recovery processes consistent with those observed in vivo. Unlike the migrating contact area, which preserves hydration by moving faster than interstitial fluid can flow, our results demonstrate that the stationary contact area can maintain and actively recover hydration through a dynamic competition between load-induced exudation and sliding-induced recovery. The results demonstrate that sliding contributes to the recovery of fluid and solutes by cartilage within the contact area while clarifying the means by which it occurs.

1. Introduction

Articular cartilage is the load-bearing, avascular tissue responsible for the near-frictionless and wear-free movement of joints. Without a direct blood supply, chondrocytes within cartilage rely on diffusive and convective processes to exchange fluids, nutrients, waste products, and signaling molecules with the bathing synovial fluid (Evans and Quinn, 2006; Garcia et al., 2003). Mechanical loading and the relative surface motion inherent to joint articulation largely control these transport processes (Holmes et al., 1980; Marouda,s 1975, 1976; Mow et al., 1992).

As a biphasic material, cartilage consists of an extracellular matrix (ECM) whose interstitial space is filled with fluid (Holmes et al., 1980). When cartilage is loaded in compression, the exudation of interstitial fluid helps carry cellular waste out of the tissue into the surrounding synovial fluid. However, the exudation process causes a time-dependent reduction in tissue thickness, stiffness, lubrication, and permeability to fresh nutrients (Ateshian, 2009; Holmes and Mow, 1990; Mow et al., 1980).

Significant attention has been devoted to elucidating how cartilage replenishes nutrient supplies following fluid exudation. Static and low frequency cyclic compression reduce solute diffusion by reducing matrix pore size and permeability (Maroudas et al., 1968). However, faster loading cycles, comparable to those associated with gait, can enhance solute transport into cartilage via a combination of advection and preferential entrapment of large solutes within consolidated interstitial spaces (DiDomenico et al., 2016; Zhang et al., 2007). Joint articulation also promotes fluid and solute recovery by intermittently exposing the 'dehydrated' cartilage surface directly to the synovial bath, permitting osmotic and diffusion-driven transport of fluid and solutes into the surface

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(Ekholm, 1955; Maroudas et al., 1968). Additionally, the fluid mechanics of joint articulation (e.g. hydrodynamic pressure) have been implicated in fluid and solute recovery and transport processes in cartilage (Gleghorn and Bonassar, 2008; Graham et al., 2017; Hou et al., 1992; Ling 1974; Moore and Burris, 2016; Wright and Dowson, 1976). However, these potential tribological consequences on cartilage fluid and nutrient recovery have proven difficult to isolate experimentally, particularly in the MCA where osmotic swelling during intermittent bath exposure is presumed to prevail.

Recently, our group has used the convergent stationary contact area (cSCA) configuration (Fig. 1A) to study these hypothetical hydrodynamic effects while eliminating confounding effects from dynamic compression or contact migration (Graham et al., 2017; Moore and Burris, 2016). Estimates for physiological sliding speeds are ~100 mm/s (Hou et al., 1992; Mow, 1969) with contact pressures between 1 and 6 MPa during daily joint use (Park et al., 2008). In our previous papers we found that sliding under contact pressures near these values (60 mm/s under 0.25 MPa contact pressure) drove marked cartilage fluid and solute recovery, as measured by in situ compression and confocal imaging of solute accumulation. Although we have shown that this purely sliding-induced recovery phenomenon, termed tribological rehydration, restores hydration, thickness, load support, lubrication, and 'nutrition' following exudation, the mechanism(s) underlying this phenomenon remain uncertain. In this study, we used intermittent resting periods between sliding bouts to map (image) the spatiotemporal evolution of solute transport from the bath into the buried contact interface as a means to elucidate the flow fields associated with tribological rehydration. In the process, we also discovered that each bout of brief sliding reversed the exudation that accompanied each static 'imaging' period, preventing net loss of interstitial fluid or pressure over the long term. Given the intermittency inherent to human movement, we think this dynamic balance between exudation and tribological rehydration may provide important insight into the links between exercise and long-term joint health (Ageberg et al., 2012; Bosomworth, 2009; Hunter and Eckstein, 2009; Manninen, 2001; Rogers et al., 2002; Urquhart et al., 2011; Williams, 2013).

2. Methods

2.1. Setup design

19 mm diameter osteochondral cores were harvested from the femoral condyles of previously frozen mature bovine stifles, stained overnight in 10 μM 5′-DTAF (Life Technologies), and washed as described previously (Graham et al., 2017). Thawed sampled were tested within 4 days of harvesting, which has been previously shown to have no effect on tribological performance when compared to freshly harvested samples (Moore and Burris, 2015). Samples clamped via the subchondral bone were affixed to a mechanical tester featuring a linearly reciprocating glass slide that samples were compressed and slid against (Moore and Burris, 2016). This testing configuration (Fig. 1A) is classified as a convergent stationary contact area (cSCA), because a portion of the total cartilage surface area is in constant contact with the glass, thereby creating 'convergent wedges' at the contact periphery. The tester was mounted adjacent to an LSM880 confocal microscope used to image the buried cSCA contact during the sliding experiments using a 20x objective (LD Plan-Neofluar 20x/0.4, Carl Zeiss Microscopy) and light inverter.

2.2. Mechanical testing

Samples were preconditioned by applying 5 N compression, sliding at 60 mm/s, then free-swelling in 1X phosphate buffered saline (PBS), with each step lasting 2 min. The loading protocol shown in Fig. 1B begins with the initial application of a static 5 N load followed by alternating periods of reciprocal sliding (2 min) at 60 mm/s over a 20-mm distance (in both forward and reverse

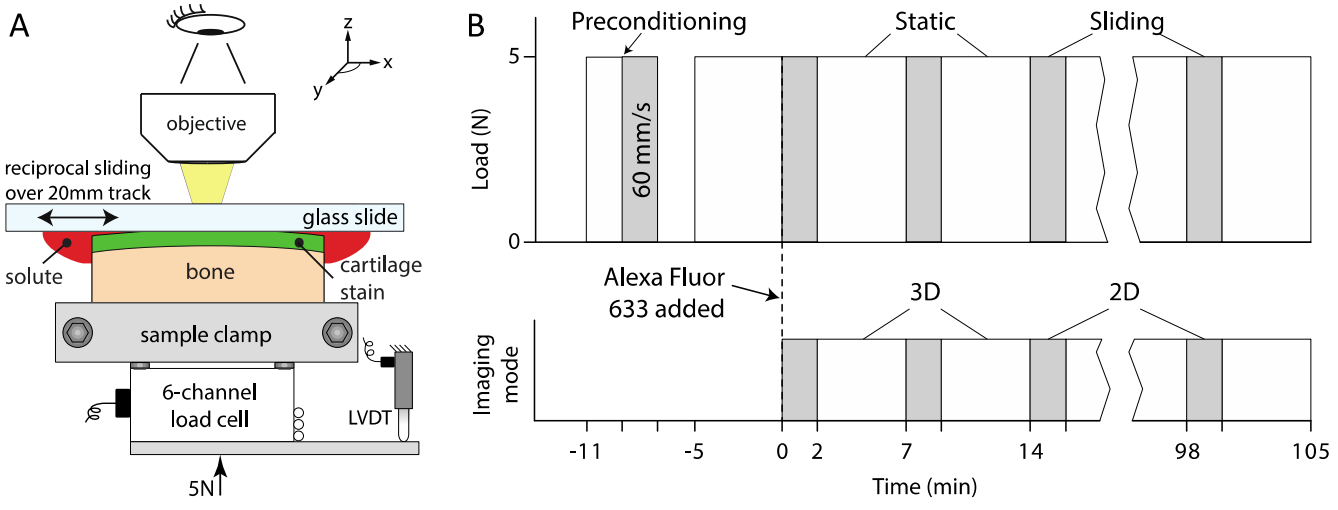

Fig. 1. Experimental design for the simultaneous collection of confocal fluorescence and tribology data during intermittent sliding in the cSCA configuration. (A) Schematic of the combined microscope and tribometer configuration. The sample remains stationary relative to the objective while the glass slide reciprocates, permitting simultaneous image and tribology data collection. The materials tester is mounted on an automated x-y stage to allow the generation of mosaic images of large areas, which is used to image the entire contact length. While the entire explant is not fully immersed in the solute solution, surface tension and adhesion to the glass cause the solution to fully envelope and bathe the cartilage. This condition is maintained even during high speed sliding. (B) Graphs depict the load applied to the sample along with the intermittent sliding and image capture conditions. Gray background indicates sliding at 60 mm/s while simultaneously collecting 2D time series of solute accumulation. White background indicates static contact while 'tiled' 3D scans were collected across the full contact length. A solution containing the small, neutral solute AlexaFluor 633 (AF633; shown in red) is used to replace the PBS bathing solution at time t = 0 min. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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