Acta Biomaterialia xxx (xxxx) xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actbio

Full length article

The modes and competing rates of cartilage fluid loss and recovery

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ARTICLE INFO

Article history:
Received 14 April 2021
Revised 10 November 2021
Accepted 11 November 2021
Available online xxx

Keywords:
Cartilage
Hydration
Free-swelling
Passive-swelling
Tribological rehydration

ABSTRACT

Cartilage loses, recovers, and maintains its thickness, hydration, and biomechanical functions based on competing rates of fluid loss and recovery under varying joint-use conditions. While the mechanics and implications of load-induced fluid loss have been studied extensively, those of fluid recovery have not. This study isolates, quantifies, and compares rates of cartilage recovery from three known modes: (1) passive swelling – fluid recovery within a static unloaded contact area; (2) free swelling – unrestricted fluid recovery by an exposed surface; (3) tribological rehydration – fluid recovery within a loaded contact area during sliding. Following static loading of adult bovine articular cartilage to between 100 and 500 μm of compression, passive swelling, free swelling, and tribological rehydration exhibited average rates of 0.11 $\pm$ 0.04, 0.71 $\pm$ 0.15, and 0.63 $\pm$ 0.22 μm/s, respectively, over the first 100 s of recovery; for comparison, the mean exudation rate just prior to sliding was 0.06 $\pm$ 0.04 μm/s. For this range of compressions, we detected no significant difference between free swelling and tribological rehydration rates. However, free swelling and tribological rehydration rates, those associated with joint articulation, were ~7-fold faster than passive swelling rates. While previous studies show how joint articulation prevents fluid loss indefinitely, this study shows that joint articulation reverses fluid loss following static loading at >10-fold the preceding exudation rate. These competitive recovery rates suggest that joint space and function may be best maintained throughout an otherwise sedentary day using brief but regular physical activity.

Statement of Significance

Cartilage loses, recovers, and maintains its thickness, hydration, and biomechanical functions based on competing rates of fluid loss and recovery under varying joint-use conditions. While load-induced fluid loss is extremely well studied, this is the first to define the competing modes of fluid recovery and to quantify their rates. The results show that the fluid recovery modes associated with joint articulation are 10-fold faster than exudation during static loading and passive swelling during static unloading. The results suggest that joint space and function are best maintained throughout an otherwise sedentary day using brief but regular physical activities.

1. Introduction

Cartilage, the multiphasic tissue responsible for the load bearing and lubrication of joints, normally comprises about 75% interstitial fluid [1]. Under fully hydrated conditions, interstitial pressure preferentially supports the load, which reduces tissue strain and friction [1–4]. While interstitial pressure performs key functional roles, it is also responsible for fluid exudation, a process that defeats interstitial hydration, pressure, and lubrication [2]. Indeed, experimental studies with cartilage explants have shown that tissue strains and friction coefficients approach 50% and 0.3, respectively, as fluid is lost over time [4–8].

The mechanics of fluid exudation are well understood, predictable, and observable even at the joint level [9–13]. Loading experiments with cadaveric human knees have shown that joint strains can reach 50% within 2 h of static loading [14]. Fortunately, this exudation process is arrested during physical activity. In one study, MRI measurements during repeated sets of knee bends showed no time-dependent joint space thinning following an initial strain of only ~5% [15]. Theoretical work from Ateshian and Wang showed that exudation effectively stops during articulation because the contact area migrates across the cartilage surface

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https://doi.org/10.1016/j.actbio.2021.11.014
1742-7061/

faster than the interstitial fluid can respond [2]. Interestingly, Coleman *et al.* showed that joint space thickness in the human knee is quite effectively preserved, thinning only by 1-5%, throughout the day [16]. Given that the average American is physically inactive or sedentary for most of the average day [17], such findings suggest that joint space thicknesses reflect a dynamic competition between load-induced exudation and movement-induced fluid recovery rather than the absolute absence of fluid flow.

The mechanics of fluid recovery by cartilage has received disproportionately less research attention than the mechanics of fluid loss. Eckstein *et al.* showed that the human knee thickened during rest following exercise at an average rate of 0.03 μm/s [15]. This recovery rate was uncompetitive with the loaded exudation rates observed at similar strains [14], which raises questions about the nature of the surfaces and interface during the recovery process. The authors attributed this slow recovery process to low permeability of the consolidated surface but another possibility is that the interface itself resisted inflow.

The nature of this surface/interface boundary is arguably the most important characteristic of exudation mechanics [18]; the two limiting cases are confined and unconfined compression [9]. In confined compression, the surface boundary is defined by zero pressure and the characteristic deformation time constant is independent of contact area; unconfined compression is instead defined by a zero flow surface boundary condition and its characteristic time constant is proportional to the contact area [9]. The same distinctions must be made for fluid recovery. In this study, ***free swelling*** is the limiting case of fluid recovery by an exposed surface with a zero-pressure boundary condition at the surface; ***passive swelling*** is the limiting case of fluid recovery within an unloaded contact area with a the zero-flow boundary condition at the surface. While it is reasonable to assume that cartilage surfaces separate during/following unloading, experimental evidence indicating that contact areas remain following unloading [19,20] and even persist under tension [21–23] refute this assumption. To date, however, the nature of these unloaded interfaces and their implications for fluid recovery dynamics remain largely unstudied.

We recently discovered another distinct mode of fluid recovery, which we call ***tribological rehydration,*** using convergent stationary contact area (cSCA)[1] sliding experiments designed to activate hydrodynamic pressurization [24]. In the case of the cSCA, sliding caused interstitial fluid recovery within the shielded contact area without any change in the applied load. Our studies of tribological rehydration consistently suggest that hydrodynamic pressures are responsible for pushing entrained fluid into the loaded contact area [24–26]. Unlike free swelling, which only rehydrates surfaces outside the contact area, tribological rehydration rehydrates surfaces within the contact area (*e.g.*, the femoral condyle and tibial plateau contact patch). Unlike passive swelling, which requires reductions in applied loads, tribological rehydration occurs at constant loads. While the phenomenon of articulation-induced fluid recovery has been widely attributed to free swelling at exposed surfaces [3,27], tribological rehydration may also represent an important contributor. However, because these modes have yet to be isolated and quantified, their potential contributions to fluid recovery within joints remain uncertain.

The preservation of joint space during physical activity is often attributed to the absence of flow [6], but we propose that the constant strains observed within articulating joints [13,28] represent a dynamic equilibrium between competing rates of fluid loss and fluid recovery. This distinction between competing flows and the absence of flow at equilibrium [6,10] may be extremely important physiologically and biomechanically given that local flows mediate solute transport, biochemistry, mechanobiology, non-equilibrium biomechanics, and other flow-dependent processes [1,3,13,29]. Furthermore, it implies that the cartilage strain behaviors typically observed during activity [13,15,16,24,27] fundamentally depend on a competition between rates of fluid loss and fluid recovery. This paper isolates and quantifies these rates for the first time using controlled cartilage explant testing under physiologically relevant stresses, strains, and sliding speeds. The results show that free swelling and tribological rehydration are disproportionately fast compared to passive swelling and suggest that periodic joint movements may represent a critical mechanism for preserving and even restoring cartilage hydration and joint space throughout a mostly sedentary typical day.

2. Methods

2.1. Materials

This study used nine mature (>18-month-old steer) bovine stifles acquired through two local sources (Herman's Quality Meats, Newark, DE and Bowman's Butcher Shop, Churchville, MD). Following thawing and joint dissection, a coring saw was used to extract 19 mm diameter osteochondral cores from the medial and lateral femoral condyles. Each sample was stored and hydrated in phosphate buffered saline (0.15 M) containing protease inhibitor (P2714, Sigma Aldrich); we refer to this solution as PBS from here on. Samples were stored at 4°C for less than 4 days prior to testing and they were tested at 23°C over a period of less than 12 h.

2.2. Instrument and measurements

The custom pin-on-disc tribometer shown in Fig. 1 was used to quantify fluid loss and recovery by measuring sample compression in real time under varying experimental conditions (loaded or nominally unloaded, static or sliding). Normal loads (F_n) were measured with a 6-channel load cell (ATI nano17, ±10 mN) and controlled using a vertical nanopositioning stage (Q-545, Physik Instrumente) with force feedback. The displacements of this stage (± 10 nm) were used to maintain constant load conditions and to track cartilage compressions (δ) and compression rates ($\dot{\delta}$) *in situ* (i.e., cartilage thickness). The load head assumed either of two orientations. The 'cartilage-on-flat' mode (Fig. 1a) was used for passive swelling and tribological rehydration characterization. In this configuration, large diameter convex osteochondral samples were mated against an impermeable glass counterface that was fixed or rotated (ω = 0 or 4.8 rad/s, respectively) depending on the experiment. The 'indenter-on-cartilage' mode (Fig. 1b) was used for free swelling measurements. In this configuration, a cylindrical porous plane-ended indenter with effectively infinite permeability was used to load and nominally unload cartilage (to a tare load) without inhibiting fluid flow out of or into the compressed surface. The cartilage surface was aligned normal to the indenter's axis of rotational symmetry using a two-axis tilt stage. Both the cartilage surface and indenter were bathed in PBS.

2.3. Fluid recovery measurements

A total of 21 osteochondral cores were used to quantify free swelling (N=7 samples), passive swelling (N=7), or tribological rehydration (N=7) rates following varying periods of fluid exudation. These recovery modes are depicted in Fig. 1. We used static recovery of cartilage under a nominal 0.1 N tare load against a porous indenter to represent ***free swelling***; this experiment tracks recovery without obstructing fluid recovery through the 'contacting' surface [30,31]. We used static recovery of cartilage in con-

[1] By definition of the cSCA, the contact diameter is smaller than the sample diameter. This creates a convergent wedge, which enables hydrodynamic pressurization during sliding.

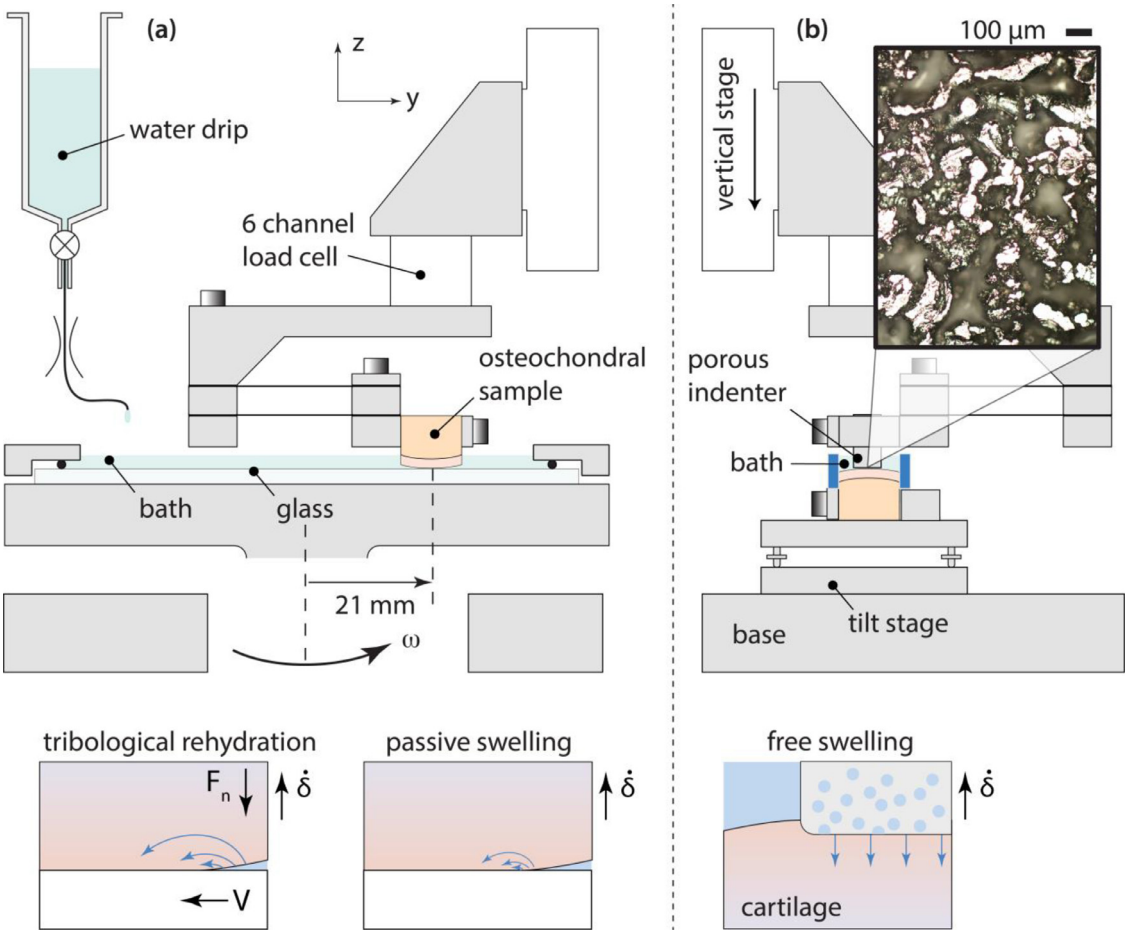

Fig. 1. Schematic of the experimental apparatus. (a) The cartilage-on-flat configuration (which generates a convergent stationary contact area, cSCA) was used for passive swelling and tribological rehydration tests. The sample was clamped via the subchondral bone and the convex cartilage surface was loaded against a glass disc 21 mm from a rotary spindle. A calibrated water drip added DI water at the same rate as evaporation to maintain osmolarity. (b) The indenter-on-cartilage configuration was used with a porous (60 μm pores, 60% solid, permeability (k) ~2500 mm^4/Ns) plane-ended stainless-steel indenter (ϕ 6 mm) for free swelling tests. The sample was mounted to a 2-axis tilt stage to align it with the indenter. Schematic sketches of tribological rehydration, passive swelling, and free swelling are shown for reference.

tact under a nominal 0.1 N tare load against impermeable glass to represent ***passive swelling***; this fixed-boundary experiment is well-controlled yet mimics the cartilage-on-cartilage passive recovery response as shown in S1. In-situ contact area measurements showed that the contact pressure during passive swelling is ~1-10 kPa (Fig. S2). We used recovery of cartilage under a maintained 5 N load against an impermeable glass surface sliding at a mean speed (V) of 100 mm/s to represent ***tribological rehydration***. The measured contact pressures were ~0.1-0.2 MPa (Fig. S2-3); for context, this is consistent with the contact pressures observed during static body weight loading of the human knee in-vivo (~0.2 MPa) [32]. The 100mm/s sliding speed was selected as it falls in the middle of the range cited for the human knee during gait [33,34]. The superficial layer fiber direction was not recorded during extraction and was randomly oriented with respect to the sliding direction; a previous study testing this effect detected no significance [25].

To start each test, samples were loaded at 1 N/s to a target of 5 ± 0.05 N. We neglected flow during each loading and unloading ramp and treated deformations during loading and unloading as effectively elastic[2]. At the target load, the sample lost fluid over time until reaching a randomized compression target between 100 and 500 μm; these targets correspond approximately to the compressions reported for the human knee after 10 to 100 min of static loading [14]. Following this point, passive and free swelling were initiated by reducing the load to 0.1 ± 0.05 N at 1 N/s. The fluid recovery rate (passive swelling and free swelling) was quantified as a function of time (after reaching the target) using the time derivative of compression measurements; we used a zero-phase filter with a five-point moving average to mitigate the effect of measurement noise on the derivative. These loss and recovery rates are based on changes in cartilage thickness (μm/s) and they are equivalent to the rate of volumetric recovery per unit contact area. We then averaged these results from the first 100 s of recovery to quantify a representative mean rate for each sample; we chose this time to i) capture temporal changes, ii) represent a physiologically relevant time scale (e.g., a walk to the water cooler), and iii) avoid excessive weighting by the decay toward zero near equilibrium.

Following exudation, tribological rehydration was initiated by sliding the disk at 100 mm/s while maintaining a constant 5 N load. The magnitudes of fluid exudation and recovery rates were quantified before and during sliding, respectively, by taking the time derivative of filtered compression data as described above. In this case, the applied load was maintained so there was no elastic recovery component. Given prior experimental evidence that exudation is approximately independent of sliding [4], net fluid recovery, which is measured, represents the difference between tribological rehydration (fluid gained) and exudation (fluid lost). To quantify the tribological rehydration rate at a given time, we added

[2] Our biphasic model [47] with typical properties gives a mean flow rate of ~50 nm/s, which indicates that ~0.25% of the 'elastic' deformation is attributable to flow

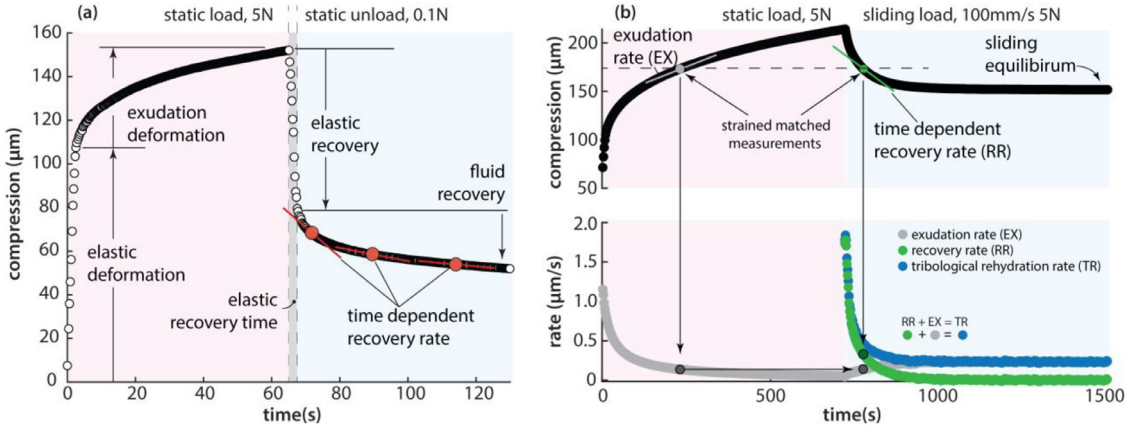

Fig. 2. (a) Methods used to quantify passive and free-swelling rates ($\dot{\delta}$), which are defined here as the rate of change in thickness or the rate of volume change per unit contact area. Following static exudation at 5 N to a target compression, load was decreased to a tare load of 0.1 N. Time-dependent passive and free swelling rates were quantified using the instantaneous slope of recovery data taken just after reaching the 0.1 N tare load. The term 'elastic' denotes active loading and unloading phases where fluid volume is approximately conserved. (b) Methods used to quantify tribological rehydration and exudation rates, also defined based on the change in thickness per unit time. Following static exudation at 5 N to a target compression, fluid recovery accompanied the onset of sliding at 100 mm/s under the constant 5 N load. The time-dependent exudation and recovery rates were quantified using the instantaneous slopes of the exudation and recovery curves as shown. The tribological rehydration (total fluid gained, blue points) rate is the sum of the strain-matched exudation (fluid lost, grey) and recovery (net fluid gained, green) rates as shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the measured recovery rate and the exudation rate for the same compression as shown for an arbitrary time point in Fig. 2b.

While there may be considerable uncertainty in the exudation rate during sliding, the confounding effect on tribological rehydration rates is minimal when recovery rates are positive; as Fig. 2b illustrates, the tribological rehydration rate only deviates significantly from the measured recovery rate due to exudation when the recovery rate approached zero. These uncertainties can become dominant when the majority of the recover signal comes from exudation. To avoid this potentially confounding effect, we only quantified tribological rehydration rates when net recovery rates were positive. An example of net exudation can be found in Fig. 4a.

2.4. Data analysis

The primary objectives of the study were to quantify mean rates of cartilage compression recovery due to passive swelling, free swelling, and tribological rehydration and identify any significant differences between these recovery modes. To detect statistical differences between average rates, we utilized one-way ANOVAs with Tukey's HSD multiple comparisons tests; the threshold for establishing statistical significance was defined as a multiplicity adjusted *p-value* of $p < 0.05$. A secondary aim was to determine if recovery rates characterized by a given mode depended on the initial exudation. These relationships were assessed using Pearson's correlation coefficients (r), with the equation of the linear fit of this data being provided to assist interpretation. All data analyses were performed using MATLAB 2019b.

3. Results

Passive swelling results are shown in Fig. 3a (green data points) for a single representative sample subjected to varying compression targets. The last 15 s of exudation (dark background) under static loading (5N) and the elastic recovery response (light background) during unloading (to 0.1N) are shown for reference. Fig. 3c and d show that passive swelling rates slowed monotonically as cartilage recovered fluid (i.e., decompression) over time; in this case, passive swelling rates decreased from an initial range of 0.10-0.25 μm/s to a range of 0.01-0.10 μm/s after 150 s.

Free swelling results are shown in Fig. 3b for a second representative sample subjected to varying compression targets (yellow data). The elastic recovery was about 50 μm for both sets of experiments regardless of the initial exudation. Fig. 3c and d show that free swelling had a first-order character similar to that of passive swelling but with faster rates; free swelling rates decreased from an initial range of 0.9 to 1.3 μm/s to a range of 0.2 to 0.4 μm/s after 150 s.

Tribological rehydration results are shown in Fig. 4a for a third representative sample subjected to varying compression targets followed by loaded high-speed sliding. The load was fixed at 5 N and the compression measurement comprised an initial elastic component (~100 μm on average) and an initial exudation component (indicated). Regardless of the initial exudation condition, this sample approached the same dynamic equilibrium (~175 μm) during sliding. The sample recovered fluid during sliding when the compression exceeded the dynamic equilibrium and it lost fluid when sliding was initiated below this target (*e.g.*, the 98 μm initial exudation case). These observations are consistent with our hypothesis that the absence of net flow at the dynamic equilibrium reflects a competitive balance between tribological rehydration (input) and exudation (output) rather than the absence of flow in an absolute sense. The amount of net fluid recovered by the sample increased with initial exudation as shown in Fig. 4b. In each of the four 'net recovery' cases[3], tribological rehydration rates were 1-2 μm/s initially and decreased over time to a final value of ~0.2 μm/s at the dynamic equilibrium (Fig. 4c). As Fig. 4c illustrates, the dynamic equilibrium represents the compression/strain for which the tribological rehydration rates equal the exudation rates. Across all samples and conditions, the mean and standard deviation for the dynamic equilibrium compression and the dynamic equilibrium inflow/outflow rates were 181 ± 64 μm (82 μm from outflow after accounting for 99 μm of elastic compression due to loading) and 0.22 ± 0.07 μm/s, respectively.

Fig. 5a shows how the mean flow rates (all samples, all conditions) vary as a function of time for the first 100 s of each recovery experiment; the shaded regions represent 95% confidence intervals for these mean values. All three modes slowed as the system

[3] We excluded the 98 μm initial exudation case from our analysis because it experienced 'net exudation' during sliding. While it is possible to quantify tribological rehydration rates despite net exudation, the measurement signal is dominated by exudation and sensitive to the uncertainty in the exudation rate.

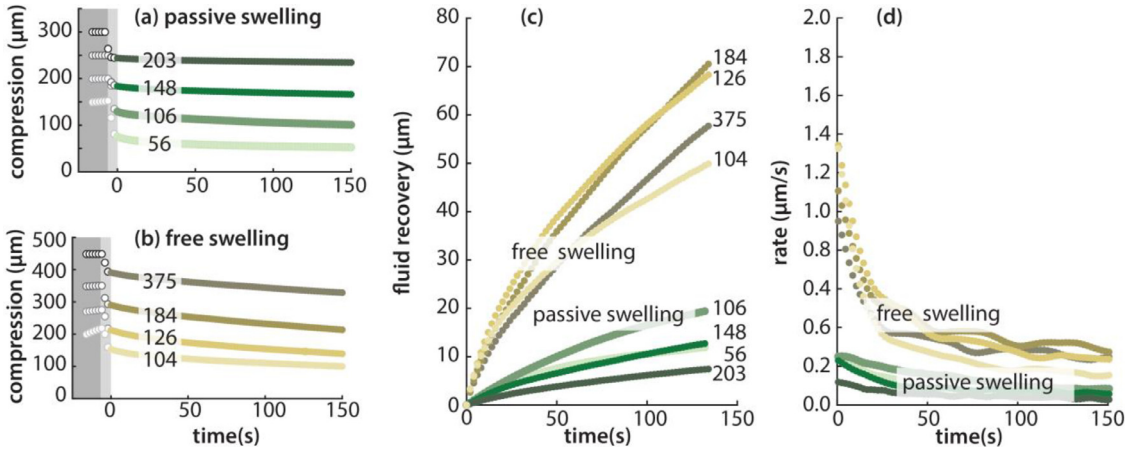

Fig. 3. Results of passive and free swelling experiments. (a) Raw end-of-compression and start-of-recovery results from passive swelling measurements for a single representative sample subjected to varying tissue compression; each experiment is labeled with its initial exudation in microns. (b) Raw compression and recovery results from free swelling measurements for a different representative sample with varying tissue compression. The exudation and elastic recovery portions of the curve are highlighted in dark and light grey zones, respectively. (c) Fluid recovery (in microns) for passive and free swelling versus time from the same two representative samples. (d) Passive and free swelling rates (in microns per second) versus time for the same two representative samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

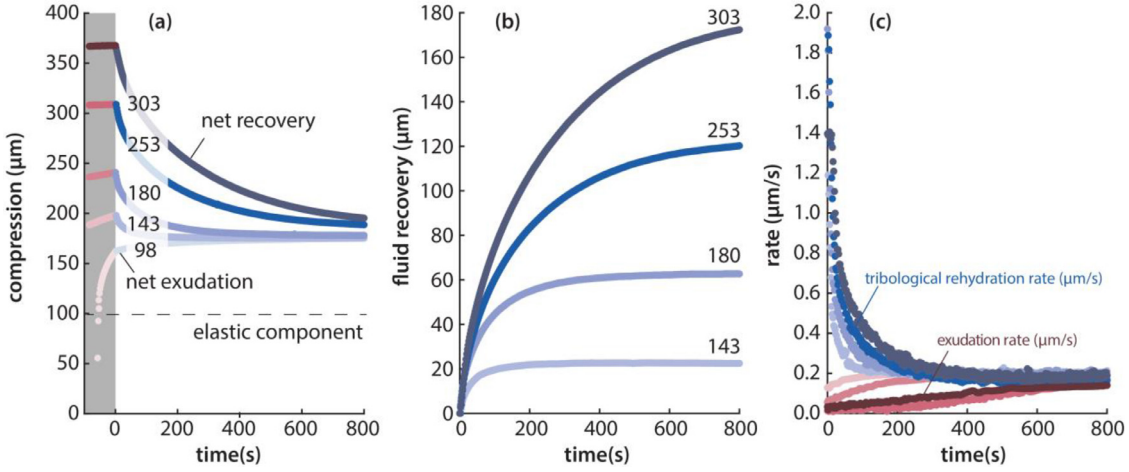

Fig. 4. Results of tribological rehydration experiments conducted with varying tissue compressions for a single representative sample. (a) Raw compression and recovery results from tribological rehydration measurements for a single representative sample subjected to different initial compressions; each experiment is labeled with the initial compression at the onset of sliding minus the elastic compression. (b) Net measured fluid recovery starting from the onset of sliding as a function of time for the same experiments. (c) Tribological rehydration rates and exudation rates for the same experiments as a function of time. The system reached a dynamic equilibrium of zero net flow when exudation (outflow) balanced tribological rehydration (inflow) at ~0.2 μm/s each.

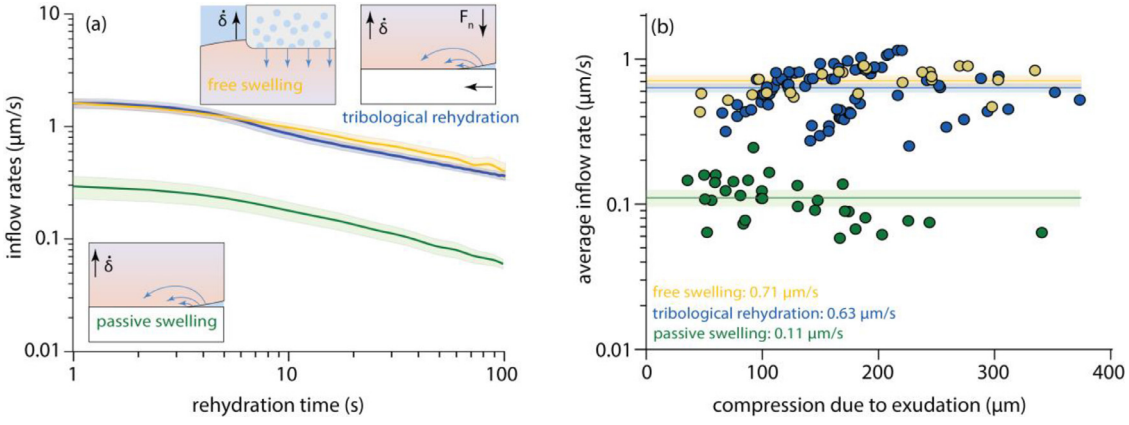

Fig. 5. Summary of fluid recovery rates for all samples. (a) Mean inflow rates from all measurements for passive swelling (green), free swelling (yellow), and tribological rehydration (blue) as a function of time for the first 100 s of recovery (shaded regions represent the 95% confidence intervals for these mean values). All three recovery rates decreased with time but tribological rehydration and free swelling rates were approximately an order of magnitude higher than passive swelling over the entire time course. (b) Average inflow rates for the first 100 s of recovery versus compression from exudation prior to recovery for all tested samples. Shaded regions represent the overall means and their 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

approached full recovery over time. Free swelling and tribological rehydration were consistently an order of magnitude faster than passive swelling. Interestingly, free swelling and tribological rehydration exhibited overlapping confidence intervals over the entire range with both rates decreasing from initial rates of >1 μm/s to 0.3 μm/s after 100 s of sliding (rehydration time).

The average inflow rate over the first 100 s of recovery is plotted for each sample as a function of compression due to exudation in Fig. 5b. For this range of testing conditions, the rates (mean ± the standard deviation) of free swelling, tribological rehydration, and passive swelling were 0.71 ± 0.15, 0.63 ± 0.22 μm/s, and 0.11 ± 0.04, μm/s, respectively. The shaded regions represent the overall means and their 95% confidence intervals. A one-way ANOVA detected significant differences among these rates. Post-hoc testing showed that free swelling and tribological rehydration rates were significantly faster than passive swelling ($p < 0.0001$); it revealed no significant difference between free swelling and tribological rehydration rates ($p = 0.16$). On average, free swelling and tribological rehydration rates were ~6 to 7-fold faster than passive swelling. We detected no significant correlation between the compression due to exudation and the tribological rehydration rate ($r = 0.11$, $p = 0.34$). However, the free swelling rate increased with compression due to exudation ($r = 0.53$, $p = 0.007$) and the passive swelling rate decreased with compression due to exudation ($r = -0.51$, $p = 0.003$).

4. Discussion

This paper defined, isolated, and quantified three distinct modes of competitive fluid recovery that cartilage has access to: *passive swelling* in nominally unloaded contact areas, *free swelling* at exposed surfaces, and *tribological rehydration* within loaded tribological contact areas. To our knowledge, these are the first direct measurements to quantify the fluid recovery rates associated with each mode.[4] Tribological rehydration rates (0.63 μm/s) and free swelling rates (0.71 μm/s) were significantly faster than passive swelling rates (0.11 μm/s) over the first 100 s of recovery. In addition, this 7-fold difference in recovery rate was generally sustained at longer timepoints. More importantly, tribological rehydration and free swelling rates were at least an order of magnitude faster than the exudation rates observed during static loading just prior to sliding (0.06 μm/s). This competitive asymmetry between fluid recovery and exudation helps explain why articulation reversed fluid loss so quickly in prior studies [24,27,33].

The specific rates reported here are, of course, limited to the conditions of the study. These rates are likely to change with sample size, load, sliding speed, and lubricant presence, among other variables [25,26,35,36]. Nonetheless, their agreement with results from more clinically relevant studies is noteworthy. Following 14 min of static loading, the mean exudation rate from intact human patellofemoral (PT) joints was 0.03 μm/s [14]. The mean passive swelling rate observed for knees of live human subjects [15] was 2 to 6-fold slower than those observed here (0.05 and 0.2 μm/s) and these differences are consistent with 2 to 6-fold larger contact areas in the PT joint [9]. Importantly, these observations are consistent with the hypotheses that joints maintain cartilage-on-cartilage contact following unloading, that the contact interface is a significant impediment to fluid recovery, and that the impediment increases with increased strain and contact area/path length. Additionally, following load-driven fluid exudation/compression within canine ankles, loaded articulation led to net fluid recovery at an estimated rate of ~1 μm/s [27], which agrees well with the free swelling and tribological rehydration rates observed here. This preliminary agreement between our results and those of more clinically relevant studies suggests that our selection of stresses, strains, and sliding speeds provide a strong degree of physiological relevance while also providing experimental control. Perhaps even more interestingly, the dynamic equilibrium strain response during tribological rehydration (~5% from fluid flow) was consistent with that observed in the knees of mobile human subjects [13,16]. Whether this consistency was fortuitous or the results of a more fundamental biomechanical response by cartilage remains to be seen. Subsequent work will aim to clarify how controllable experimental variables such as load, stress, and sliding speed influence the recovery process and dynamic equilibrium response.

The observation that joints maintain interstitial hydration and pressure during activity has been explained previously by the idea that the contact area migrates across the cartilage surface faster than the interstitial fluid can respond [2,10]. This absence-of-flow paradigm is useful, particularly under equilibrium conditions, but it fails to provide insight into non-equilibrium dynamics. For example, it cannot explain the observation that cartilage in the human knee gradually thins toward a dynamic equilibrium during the first 30 min of walking [13]. To resolve this outstanding issue, we propose the competitive rates paradigm illustrated by Fig. 6. During static loading, our observations and those of from the human patellofemoral (PF) joint [14] suggest exudation rates on the order of 0.05 μm/s (Fig. 6c). Unloading the joint statically leads to comparable passive swelling rates of 0.03 μm/s according to measurements with living human subjects [15] (Fig. 6b). During joint articulation, however, our results suggest that recovery rates are much faster due to the combined effects of tribological rehydration inside the contact (0.63 μm/s) and free swelling (0.71 μm/s) at free surfaces outside the contact (Fig. 6c). Linn's measurements of exudation during static loading and joint space thickening during loaded and unloaded articulation are consistent with this paradigm and the rates measured here [27].

Our competitive-rates paradigm helps explain why Paranjape *et al.* [13] observed overall fluid exudation despite contact migration during walking. Walking from a fully hydrated state (the intended initial condition of the study), where the initial strain is assumed to be 0%, requires net exudation. As net exudation occurs, however, exudation rates decrease and recovery rates increase until meeting at the dynamic equilibrium (~5% strain is typical [13,15,16,27]). In general, one can expect net fluid loss whenever strains are below their dynamic equilibria and net fluid recovery otherwise. Linn showed this effect directly. He observed either net fluid loss or net fluid recovery to the same dynamic equilibrium when he articulated canine ankles from a fully recovered state or from a consolidated state, respectively [27]. The same effect is evident in Fig. 4; depending on the initial exudation, the sample exhibited either net exudation or net recovery during sliding, as appropriate, until reaching a consistent sample-dependent dynamic equilibrium compression. An important and unstudied practical question is whether and how quickly joint space is restored during walking following static loading (*e.g.* an hour at a standing desk). Our observations suggest that as little as a few minutes of walking might restore joint space following an hour or more of continuous static loading. This hypothesis can be tested with existing methods and the results may have significant clinical and occupational implications.

Beyond clinical implications, these results help answer longstanding questions about the conditions of the contact interface following nominal unloading. Biphasic analysis assumes that the cartilage contact area is effectively impermeable [10] but this neglects the possibility that cartilage absorbs fluid from the interfacial gaps created by its unusually rough surfaces [37]. The latest theoretical studies support the impermeable interface assump-

[4] We aim to make these data available in a future data note.

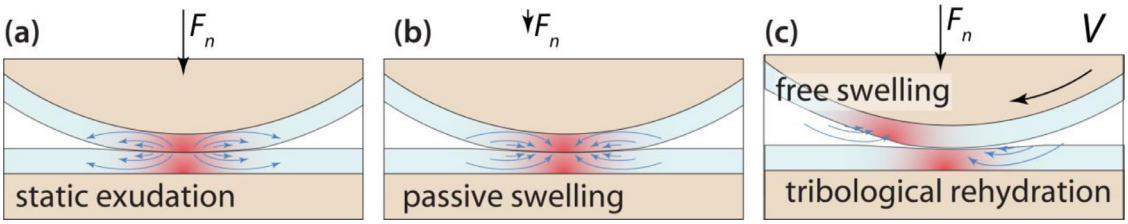

Fig. 6. An illustration of the competing rates paradigm for describing cartilage fluid retention in the joint. (a) Exudation occurs during loading and the rates of fluid loss decrease with increasing contact area [9]. (b) Passive swelling occurs during static conditions within the contact area. (c) Free swelling and tribological rehydration occur during articulation outside and inside the contact area, respectively.

tion [38–41]. In particular, a study by Liao *et al.* showed that exudation rates in realistically rough cartilage contacts were only slightly greater than those of an impermeable interface because compression of individual asperities reduces surface roughness, the size of interfacial gaps, and the interfacial permeability [39]. Using only basic contact mechanics arguments, Klein previously reached a similar conclusion that 'under the pressures of living joints, the surfaces of the sliding articular cartilage are expected to conform smoothly and uniformly to each other, presumably down to separations of order nanometres' [42]. Because both arguments are based on load-induced deformation, it is reasonable to expect asperity decompression, increased interface permeability, and high recovery rates following nominal unloading. However, our results are inconsistent with this expectation; the observation that passive swelling rates were only ~17% the free swelling rates suggests that the nominally unloaded (0.1 N tare load) contact area remained resistant to fluid inflow and that the unloaded asperities remain highly compressed. The low interfacial permeability (coupled with long fluid path lengths) following joint unloading helps explain the otherwise surprising fact that cartilage contact areas remain large following unloading [19,20] (see Fig. S2) and even persist under tension [21–23].

Extremely thin interfacial gaps can be expected to slow exudation and passive swelling while amplifying hydrodynamic pressures and promoting tribological rehydration. Thus, it appears that movement-induced recovery is favored by design over static recovery. Interestingly, a recent theoretical study [43] showed that tribological rehydration is driven by hydrodynamic pressures and actually anticipated the magnitude of the tribological rehydration rates observed here. Given its hydrodynamic origins, we were surprised that tribological rehydration rates at loaded low permeability interfaces were comparable to and even statistically indistinguishable from free swelling rates. It is possible that this is mere coincidence. Another more likely possibility is that hydrodynamic pressures only serve to push fluid into the low permeability interface [25,44] and that osmotic swelling pressures ultimately regulate fluid uptake rates (as they do for free swelling). This hypothesis suggests that tribological rehydration rates will be limited by the lesser of the hydrodynamic transport rate (into the interface) or the free swelling rate. In the future, we will vary sliding speed (the driver of hydrodynamic transport) and contact stress and/or osmolarity (a driver of free swelling) to test this hypothesis more directly.

This study has several important limitations. While the conditions of this study were selected for their physiological relevance, no single set of conditions can represent the diversity of conditions in-vivo [45]; in particular, the effects of load, speed, and material properties remain untested. The only effects we tested here were those of time and strain. Free swelling rates increased with strain due, presumably, to increased osmotic pressure (Fig. 5b). Passive swelling rates slowed with increased strain; in this case, the favorable effect of increased osmotic pressure appears to have been offset by the competing effects of increased contact area/path length, increased asperity compression, and decreased interface permeability. Lastly, we used a smooth glass surface or a porous steel indenter as the mating surface depending on the experiment. Clearly, neither surface approximates the native cartilage surface. While migration makes tribological rehydration impossible to isolate in cartilage-cartilage contacts, we were able to test unloaded (passive) recovery of cartilage-on-cartilage contacts. The results in Fig. S1 show that passive swelling occurred at about the same rate whether cartilage was mated against glass or another cartilage surface; thus, cartilage appears to be approximately impermeable from the passive swelling point of view, which explains the slow recovery during static unloading in-vivo [15]. We will study this effect in detail in a future study. In addition, the porous indenter is a concern due to the stress concentrators and the potential for surface damage. We repeated passive swelling measurements after porous indentation and observed no effect from indentation (Fig. S1). Additionally, we found no difference in the free swelling rates based on measurements with a porous indenter and a porous flat disc (not shown).

In summary, this study provides several important joint biomechanics insights. First, it provides evidence that dynamic compression/strain equilibria in cartilage reflect a balance between fluid loss and recovery rather than the absence of fluid flow. Second, it shows that movement-induced fluid recovery rates (due to direct surface exposure and tribological rehydration within the contact) are 7-fold those of passive swelling (due to nominal unloading) at the tested conditions. Third, it demonstrates that interfacial permeability is extremely low, even in nominally unloaded contacts, and suggests that low interface permeability may be the key feature underlying competitive asymmetry between movement-induced fluid recovery and load-induced fluid loss (exudation would be far faster without significant resistance to interfacial flow). Fourth, it presents a new competitive rates paradigm with which to interpret these empirical results. In a recent study, for example, we used this competing rates paradigm with strain measurements from small range-of-motion MCA experiments to deduce that free swelling rates were likely ~1 μm/s [46]; this study validates that deduction. This paradigm also provides a theoretical basis for anticipating dynamic equilibrium strains and loss or recovery rates during walking [13] and other activities. Finally, and most importantly, it suggests that brief but regular physical activity may be the most effective way to maintain maximum joint space thickness and cartilage function throughout an otherwise sedentary day.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge funding from the NSF Biomaterials and Mechanobiology Program under awards: 1635536 and 1937493.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2021.11.014.

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