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Comparison of friction and wear of articular cartilage on different length scales

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ABSTRACT

The exceptional tribological properties of articular cartilage are still far from being fully understood. Articular cartilage is able to withstand high loads and provide exceptionally low friction. Although the regeneration abilities of the tissue are very limited, it can last for many decades. These biomechanical properties are realized by an interplay of different lubrication and wear protection mechanisms. The deterioration of cartilage due to aging or injury leads to the development of osteoarthritis. A current treatment strategy focuses on supplementing the intra-articular fluid with a saline solution containing hyaluronic acid. In the work presented here, we investigated how changing the lubricating fluid affects friction and wear of articular cartilage, focusing on the boundary and mixed lubrication as well as interstitial fluid pressurization mechanisms. Different length and time scales were probed by atomic force microscopy, tribology and profilometry. We compared aqueous solutions with different NaCl concentrations to a viscosupplement containing hyaluronic acid (HA). In particular, we found that the presence of ions changes the frictional behavior and the wear resistance. In contrast, hyaluronic acid showed no significant impact on the friction coefficient, but considerably reduced wear. This study confirms the previous notion that friction and wear are not necessarily correlated in articular cartilage tribology and that the main role of HA might be to provide wear protection for the articular surface.

1. Introduction

Articular cartilage is an exceptional biological material that covers the end of bones and forms the intercalating surfaces in joints. Its main function is to withstand high loads and to provide low friction, in particular while tolerating permanent changes of moving direction (Athanasiou et al., 2013). In cartilage, low friction and good wear protection are not the result of a single lubrication mechanism: rather, the mechanism adapts to changing load, shear stress or sliding rate. At low sliding speeds in the presence of a molecularly thin fluid film, the tribological properties are governed by the interactions between contact asperities. This is known as the boundary lubrication regime. The very efficient boundary lubrication mechanism in cartilage is mediated by lubricants such as PRG4 (proteoglycan 4 or lubricin), hyaluronic acid and SAPL (surface-active phospholipids) present at the articular surface (Chang et al., 2014; Coles et al., 2010; Seror et al., 2015). Another very important lubrication mechanism is the interstitial fluid pressurization (Athanasiou et al., 2013). Cartilage can be described as a triphasic material composed of: (i) the solid, porous network composed of collagen type II and charged proteoglycans, (ii) a fluid phase and (iii) ions. The fluid phase is the main component of cartilage (70–80%). The negatively charged proteoglycans draw water into the matrix ("Donnan effect") and cause the network to swell. Together with the low hydraulic permeability of cartilage, this produces a high interstitial fluid pressure which is counterbalanced by the collagen network. If cartilage is loaded, most of the load is supported by the fluid and not by the solid matrix (Ateshian, 2009; Bonnevie et al., 2011; Forster and Fisher, 1999). If the contact continuously migrates over the cartilage surface, the friction force remains low and constant (Caligaris

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and Ateshian, 2008; Chan et al., 2010). However, if one region is loaded permanently, the fraction of load supported by the fluid decreases as the fluid is squeezed out of the matrix. Such a static contact causes an increase of the friction coefficient from an initial value to a plateau friction coefficient (Mow et al., 1984; Soltz and Ateshian, 1998). The time until equilibrium is reached depends on the contact area and the permeability of the tissue (Mow et al., 1980; Park et al., 2004).

Although healthy cartilage lasts for decades without losing its function, with advanced age it often starts to deteriorate leading to the development of osteoarthritis. Osteoarthritis can have a genetic basis, but may also be induced by injuries or wear caused by age or malalignment of the joint (Andriacchi et al., 2009; Athanasiou et al., 2013). The onset of the disease is associated with a loss of mechanical properties on the micro- and macroscale (Wilusz et al., 2013). These changes can be attributed to a decrease in proteoglycan content and an increased fraction of water in the tissue (Malemud, 1991), which alters the interstitial fluid pressure and therefore directly the frictional properties. Furthermore, the molecular weight and the concentration of hyaluronic acid in the synovial fluid decrease. This reduces the wear resistance capability and can lead to further damage, also under normal loading conditions (Dahl et al., 1985).

As it is not yet possible to artificially recreate cartilage, most of the treatment strategies for osteoarthritis focus on pain reduction and maintenance of joint functionality. One such strategy are intra-articular hyaluronic acid injections (Felson et al., 2000; Pelletier et al., 2001), which are believed to increase the viscosity of synovial fluid back to its original level (viscosupplementation) and therefore improve the joint function and diminish pain (Gigante and Callegari, 2011).

One of the many commercially available viscosupplements is Sinovial[1] (Humantis, Köln, Germany). It consists of 0.8% hyaluronic acid with a molecular weight between 800 and 1200 kDa in a physiological sodium chloride solution (Gigante and Callegari, 2011). A meta-study investigating the effect of Sinovial claims that 3–5 weekly injections can reduce the pain in the joints and disabilities. However, controversial results on the impact of viscosupplementation can be found in the literature. Many studies show that intra-articular injections can help relieve the symptoms, also when placebos such as saline are injected. In some studies, almost 80% of the pain relief is accounted for by the placebo effect. Although its benefits are disputed, viscosupplementation with hyaluronic acid is regularly used during treatment of osteoarthritis and is still beneficial for some patients due to the reduction of pain.

Here, we addressed the mechanistic principle of how Sinovial influences lubrication, wear and frictional properties of articular cartilage in the boundary lubrication regime on the micro- and macroscale. We chose two complementary methods: atomic force microscopy (AFM) and a rotational tribology setup (Boettcher et al., 2014). Owing to the small contact area, the AFM allows to probe boundary lubrication (Park et al., 2004) whereas with the static contact of the tribometer the effect of the interstitial fluid pressurization can be investigated (Caligaris and Ateshian, 2008). We compared Sinovial with aqueous solutions containing different NaCl concentrations and evaluated their lubricating abilities. Finally, lubricant dependent resistance against wear was characterized using AFM and a profilometer. The different investigated lubricants showed no significant effect on the friction coefficient, but Sinovial did considerably improve the wear protection in cartilage. It is very likely that another viscosupplement containing hyaluronic acid in a similar concentration and molecular weight would lead to comparable results.

[1] Sinovial was chosen randomly for our study and we did not receive any kind of funding from Humantis.

2. Results and discussions

2.1. Surface characterization

First, we started with the characterization of the cartilage surface. We imaged the surface of freshly prepared ovine cartilage samples with an atomic force microscope in contact mode using a cantilever with a sharp tip (Fig. 1A, B and C). Directly after preparation, the samples were incubated in the lubricant of interest. As lubricants, we chose ddH_2O, 154 mM NaCl solution (physiological concentration) and 2 M NaCl solution. The cartilage network was clearly visible for all lubricants. No deposits or damage of the structure were observed. With increasing salt concentration, the network appeared denser (Fig. 1). This can be explained with changes of the electrostatic shielding of the proteoglycan charges with the ionic strength. Cartilage samples incubated in 2 M NaCl shrink due to loss of water whereas the volume of cartilage in ddH_2O increases (Ateshian et al., 2003; Eisenberg and Grodzinsky, 1985; Parsons and Black, 1979). A similar effect can be observed in osteoarthritis: The amount of water in the tissue increases in the early (often subclinical) stages of the disease (Eckstein and Wirth, 2011). The aggregation ability and the concentration of proteoglycans decrease, which in combination with the loosening of the collagen network leads to more free interstitial space filled with water (Athanasiou et al., 2013). Thus, the network in the early stages of the disease might bear resemblance to the structure of healthy cartilage incubated in ddH_2O observed by us in this study.

In addition, we imaged the surface of fresh, healthy cartilage with a profilometer (Fig. 1D). Owing to the larger scale of the image (800 μm), the individual fibers were not visible, but a smooth surface without any signs of wear was observed (Fig. 1D).

2.2. AFM-based friction force microscopy

In the next step, AFM-based friction force measurements were performed using a rectangular cantilever with a polystyrene sphere attached (Fig. 2A, inset). Articular cartilage samples were freshly prepared, anchored in a custom-built fluid cell and incubated in one of the four lubricants: ddH_2O, 154 mM NaCl, 2 M NaCl or the viscosupplement Sinovial (physiological NaCl concentration). The contact pressure during an AFM experiment can be estimated as several 100 Pa. For the parameters used here (see Section 4), we can estimate using the formula given in Park et al. (2004) (p.1684) that the interstitial fluid pressure will relax on the timescale of several ms. Therefore we can assume that only boundary lubrication is probed during an AFM experiment (Coles et al., 2008; Zeng, 2013).

Trace and retrace were recorded (Fig. 2A) and averaged to obtain the friction force F_R (Fig. 2B) and the friction coefficient μ (see Section 4 for more details on the evaluation). In Fig. 2C the velocity dependence of μ in the different lubricants is shown. No dependence on scan velocity was observed for any of the lubricants, as expected for the boundary lubrication regime (Coles et al., 2008). The lowest friction coefficients were measured in ddH_2O whereas the highest μ corresponds to the measurements in 2 M NaCl, and μ increased with increasing salt concentration. The p values obtained from Tukey test indicate that the difference in μ between ddH_2O and 2 M NaCl was significant for all velocities ($p < 0.005$). We observed a significant difference in the friction coefficient between a physiological NaCl concentration and 2 M NaCl for the two intermediate velocities ($v = 1$ Hz: $p = 0.005$, $v = 2$ Hz: $p = 0.038$). The friction coefficient measured for Sinovial was significantly larger for the lowest sliding speed compared to ddH_2O ($p < 0.01$). For the lowest speed there was also a significant difference in μ between ddH_2O and physiological NaCl concentration. Other differences were not significant (see Supplemental material). These results can be explained considering boundary lubrication,

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