Biotribology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biotribology

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

Quantification of cartilage wear morphologies in unidirectional sliding experiments: Influence of different macromolecular lubricants

Kathrin Boettcher[a], Benjamin Winkeljann[a], Tannin A. Schmidt[b], Oliver Lieleg[a,*]

[a] Department of Mechanical Engineering, Munich School of Bioengineering, Technische Universität München, Boltzmannstraße 11, 85748 Garching, Germany
[b] Schulich School of Engineering, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada

ARTICLE INFO

Keywords:
Wear
Confocal microscopy
Topography
Mucin
Lubricin
Hyaluronic acid

ABSTRACT

Quantifying surface damage on articular cartilage after exposure of the tissue to extreme or prolonged mechanical stress is not only relevant for evaluating clinically relevant alterations, e.g. when the physiological lubrication mechanisms fail, but also useful for assessing the suitability of artificial cartilage replacement materials, implants or synovia-mimetic lubricants. Here, we establish a systematic quantification method for cartilage wear formation which is based on optical profilometry – a variant of confocal microscopy. With this approach, we compare three different macromolecular lubricants, i.e. solutions containing either hyaluronic acid, lubricin or porcine gastric mucin. Depending on the counter material used for tribological testing and the macromolecule used for lubrication, we detect different types of tissue damage which we quantify with suitable topographical parameters. In our setup, mucin solutions outperform the other two lubricants: when using mucin solutions for lubrication, we do not find any signs of topographical alterations on the cartilage surface. Our results underscore the supreme protective abilities of mucin solutions - even on biological surfaces where they do not occur physiologically.

1. Introduction

Joints in the human body exhibit ultra-low friction and little wear – sometimes for a life time. This high durability can be mainly attributed to both articular cartilage, the tissue lining the joint surfaces, and synovial fluid within the joint. The interplay between the solid and fluid phase of articular cartilage in particular contributes to its outstanding material properties [1]. However, changes in the mechanical properties of cartilage or alterations in the surface topography of the tissue can lead and/or contribute to discomfort and diseases such as osteoarthritis [2–4].

Whereas the composition of the cartilage matrix and the function of the individual cartilage components are mostly well understood, the interplay between the cartilage tissue and the molecular components of the synovial fluid is still not fully clear. In particular, it remains an open question why the cartilage surface can resist wear over several decades. A variety of macromolecules are used to lubricate cartilage tissue in vivo: Early work on cartilage tribology often refers to “synovial mucin” as a component of the synovial fluid [5,6]. With the development of improved purification methods, it was shown that the synovial fluid contains hyaluronic acid as well as the mucinous glycoprotein lubricin [7,8]. HA bound on the surface of cartilage tissue was suggested to entangle with phospholipids [9] and smaller proteins, e.g., lubricin [10,11], to form a surface-anchored protective layer [12,13]. There are several studies reporting the superior boundary lubrication potential of those multi-component surface layers [10,13–18], yet there are only very few studies reporting on their wear protection abilities [14,15]. In contrast, the role of HA in boundary lubrication is still discussed [12,19,20], but HA was suggested to be important for the wear resistance of the tissue [21,22].

On the surfaces of epithelial tissues such as the cornea [23], oral cavity [24], gastrointestinal [25] and vaginal tract [26], mucus, a biopolymer-based hydrogel, serves as a protective layer preventing both microbial attack and mechanical damage to the tissue. The main component of mucus is mucin [27], a highly glycosylated glycoprotein. Mucins reduce friction [28,29] on various surfaces, and this ability is based on a combination of two effects: “hydration lubrication” [30] and “sacrificial layer mechanism” [31]. Porcine gastric mucin (PGM) combines the molecular weight range of synovial HA (~1–7 MDa [32,33]) with the high glycosylation density of lubricin (Fig. 1). Whereas lubricin possesses a hemopexin (PEX)-like domain, through which lubricin is thought to bind to cartilage surfaces [34], mucin is known to adsorb to a broad variety of hydrophobic and hydrophilic surfaces [35]. Different from lubricin, mucins can be purified comparably easily and

* Corresponding author at: Professur für Biomechanik, Technische Universität München, Boltzmannstraße 11, 85748 Garching, Germany.
E-mail address: oliver.lieleg@TUM.de (O. Lieleg).

http://dx.doi.org/10.1016/j.biotri.2017.06.002
Received 17 March 2017; Received in revised form 17 May 2017; Accepted 12 June 2017

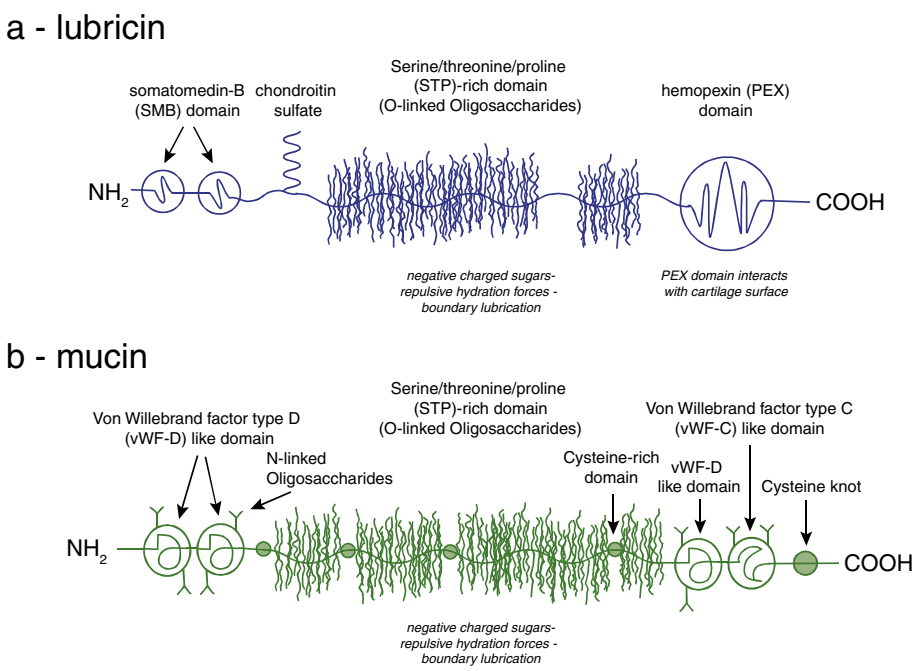

Fig. 1. Schematic structures of lubricin and porcine gastric mucin (PGM). The two glycosylated proteins share a lot of similarities but also exhibit distinct differences. Mucin monomers are secreted with a high molecular weight of around 641 kDa [37] and mucin physiologically occurs in oligomeric form with molecular weights in the MDa range. In contrast, lubricin monomers are with 227–345 kDa [38] comparably smaller. The schematic drawings are based on the following sources: lubricin [39], mucin [40].

in larger amounts [36]. However, whether mucins can prevent tissue damage in cartilage tribology has not been tested yet.

The protective properties of synovial fluid components have previously been analyzed on mica surfaces by investigating wear tracks and delamination of mica by visual examination [16,18,22]. Studies, that directly assess wear on cartilage specimens, mostly report the cartilage surface roughness as the only quantitative parameter [41–43]. To our knowledge, there are only few studies using a quantitative approach on the basis of a broader range of parameters following ISO 25178-2 [2,3,44]. Two of those studies evaluate changes in the surface topography of articular cartilage and focus on surface alterations owing to osteoarthritis. There are, however, recent studies on engineering materials (iron and steel) where different wear features are identified by employing optical measurements [45] and quantifying the obtained images with ISO parameters [46]. Traditionally, and in artificial joint prosthesis research, wear generation is characterized by determining the loss of mass, e.g., by an analytical microbalance, after a tribological measurement. This approach typically returns results with much higher accuracy than an optical inspection of the material surface. In biotribology, however, where surface damage of soft tissue not necessarily leads to erosive/abrasive wear, optical quantification methods may be more sensitive than traditional mass quantification techniques. Especially such imaging techniques which avoid mechanical contact with the sample should be very suitable, since they do not introduce artifacts into the obtained images; in contrast, contact based imaging techniques are probably less ideal since they can damage the surface of the soft tissue during imaging.

Here, we present a quantification method for tissue damage on articular cartilage based on confocal microscopy. We demonstrate that different topographical parameters a suitable for identifying specific types of tissue damage, e.g. scratches, abrasive wear or micropitting. These parameters are validated by perfoming measurements not only with cartilage tissue but also with common technical materials such as PTFE and steel. Furthermore, our results show that different macromolecular lubricants, i.e., HA, lubricin and mucin, influence wear formation on cartilage tissue in different ways.

2. Experimental section

2.1. Sample preparation

Knee joints from 3 to 6 month old lambs were obtained from local butchers and stored at − 20 °C until further usage. On the day of experiments, osteochondral cylinders with a diameter of 5.5 mm were drilled out of the trochlear groove as described in Boettcher et al. [47]. The harvested samples were incubated for at least 0.5 h in PBS (Dulbecco's PBS, Lonza, Switzerland) to ensure identical initial sample conditions and afterwards incubated in the designated lubricant for 1 h prior to a measurement. All measurements were performed and repeated on samples from at least two different animals.

2.2. Lubricants

The following lubricants were used throughout this study: PBS, or 0.1% (w/v) solutions of hyaluronic acid, mucin and lubricin, respectively, in PBS. The mucin concentration was chosen based on previously published data reporting lubrication of a 0.1% mucin solution between PDMS and steel [28], and lubricin was used at the same concentration, accordingly. All solutions were prepared in PBS (pH 7.4, Dulbecco's PBS, Lonza, Switzerland). PBS was chosen, since its pH-value fits well into the range of the pH-value of synovial fluid, both, in healthy and in arthritic joints [48]. Furthermore, at neutral pH, mucin solutions remain in a liquid state - an important aspect when considering mucins as tribosupplements.

Hyaluronic acid (HA) with a molecular weight of 2–2.4 MDa (Hyaluronic acid sodium salt from Streptococcus equi.) was purchased from Sigma Aldrich, USA.

Bovine lubricin was purified from fresh skeletally mature bovine knee joints, as described previously [49]. Briefly, cartilage discs were cultured in Dulbecco's Modified Eagle's Medium and purified using salt gradient diethylaminoethoanol anion exchange chromatography. The purity of the solution was confirmed using 3–8% Tris-Acetate SDS-PAGE followed by Simply Blue protein stain and densitometry analysis.

Porcine gastric mucin MUC5AC was purified as described previously [36] with the exception that the cesium chloride density gradient ultracentrifugation was omitted. Manual purification of porcine gastric mucin is necessary as commercial MUC5AC has strongly altered