Journal of Biomechanics 47 (2014) 659–666

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Interaction of lubricin with type II collagen surfaces: Adsorption, friction, and normal forces

Debby P. Chang [a,b], Farshid Guilak [a,c,d], Gregory D. Jay [e], Stefan Zauscher [a,b,c,*]

[a] Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC 27708, USA
[b] Center for Biologically Inspired Materials and Material Systems, Duke University, Durham, NC 27708, USA
[c] Center for Biomolecular and Tissue Engineering, Duke University, Durham, NC 27708, USA
[d] Department of Orthopaedic Surgery, Duke University, Durham, NC 27710, USA
[e] Department of Emergency Medicine, and Brown University, Providence, RI 02903, USA

ARTICLE INFO

Article history:
Accepted 26 November 2013

Keywords:
Colloidal probe microscopy
Lateral force microscopy
Atomic force microscope
Friction
Tribology
PRG4
Wear
Glycoproteins
Collagen
Type II collagen
Lubricin
SAMs
Boundary lubrication

ABSTRACT

One of the major constituents of the synovial fluid that is thought to be responsible for chondroprotection and boundary lubrication is the glycoprotein lubricin (PRG4); however, the molecular mechanisms by which lubricin carries out its critical functions still remain largely unknown. We hypothesized that the interaction of lubricin with type II collagen, the main component of the cartilage extracellular matrix, results in enhanced tribological and wear properties. In this study, we examined: (i) the molecular details by which lubricin interacts with type II collagen and how binding is related to boundary lubrication and adhesive interactions; and (ii) whether collagen structure can affect lubricin adsorption and its chondroprotective properties. We found that lubricin adsorbs strongly onto denatured, amorphous, and fibrillar collagen surfaces. Furthermore, we found large repulsive interactions between the collagen surfaces in presence of lubricin, which increased with increasing lubricin concentration. Lubricin attenuated the large friction and also the long-range adhesion between fibrillar collagen surfaces. Interestingly, lubricin adsorbed onto and mediated the frictional response between the denatured and native amorphous collagen surfaces equally and showed no preference on the supramolecular architecture of collagen. However, the coefficient of friction was lowest on fibrillar collagen in the presence of lubricin. We speculate that an important role of lubricin in mediating interactions at the cartilage surface is to attach to the cartilage surface and provide a protective coating that maintains the contacting surfaces in a sterically repulsive state.

1. Introduction

In healthy joints, articular cartilage provides an extremely efficient load-bearing surface and exhibits extraordinary lubrication and wear (Ateshian, 2009; Katta et al., 2008; Mow et al., 1992; Swanson, 1979). The superficial zone of articular cartilage and its thin, amorphous outermost layer, the lamina splendens, comprise the bearing surface for joint contact. This superficial zone consists of a mostly tangentially arranged collagen fibrillar network (Jeffery et al., 1991), and the adhesion of molecules to the cartilage surface is believed to contribute to its boundary lubrication properties (Chan et al., 2011a). Damage to this superficial zone, or absence of lubricating factors, may cause a cascade of mechanical and biological events that can lead to irreversible wear and joint disease such as osteoarthritis (Neu et al., 2010; Saarakkala, 2010 #33).

Lubricin, also called superficial zone protein (SZP) or proteoglycan-4 (PRG4), is found in the synovial fluid and also on the superficial zone of the articular cartilage, and is believed to contribute to the lubrication, wear resistance, and anti-adhesive properties of cartilage (Elsaid et al., 2005; Jay et al., 2001; Rhee et al., 2005; Schumacher et al., 1994; Swann et al., 1981). Lubricin is composed of 1404 amino acids, with a somatomedin-B (SMB)-like domain and a hemopexin (PEX)-like domain at its N- and C-terminal ends, respectively (Rhee et al., 2005). The hydrophilic center domain of lubricin is heavily glycosylated and partially capped with negatively charged sialic acid. Recent experiments confirmed that to function as an effective boundary lubricant, lubricin must adhere to the surface (Chang et al., 2009; Chang et al., 2008; Zappone et al., 2007). However, the details of lubricin binding to cartilage surfaces are still largely unknown (Nugent-Derfus et al., 2007).

Recent immunolocalization studies suggest that lubricin binds to the articular cartilage surface with its C-terminal end (Jones et al., 2007), which contains a hemopexin-like domain. This domain is conserved on many matrix metalloproteinases (MMPs), such as collagenases, where it mediates binding to native, triple

* Corresponding author at: Duke University Department of Mechanical Engineering and Materials Science, 144 Hudson Hall, P.O. Box 90300, Durham, NC 27708, USA. Tel.: +1 919 660 5360; fax: +1 919 660 5409.
E-mail address: zauscher@duke.edu (S. Zauscher).

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http://dx.doi.org/10.1016/j.jbiomech.2013.11.048

helical collagen (Murphy and Knauper, 1997; Perumal et al., 2008; Tam et al., 2002), but not on denatured collagen (Tam et al., 2002). This finding suggests that besides non-specific interaction with the cartilage surface, binding to collagen is potentially important for anchoring lubricin to the cartilage surface. Type II collagen is the predominant type of collagen in articular cartilage, where it self-assembles into large, fibrillar aggregates and adopts a complex degree of structural organization (Mow et al., 1992; Sokoloff, 1978). A monomeric type II collagen consists of three polypeptide strands, intertwined into a triple helical structure. Each triple-helix associates with its neighbors and forms microfibrils that are staggered regularly apart at a distance of 67 nm (Lehninger et al., 2000).

We hypothesized that lubricin has specific binding affinity to collagen II where it binds preferentially to triple helical collagen and mediates adhesion and friction. To test this hypothesis we used colloidal probe microscopy (CPM) to examine the conformational and tribological properties of lubricin on collagen surfaces to further understand the interaction and function of lubricin as a boundary lubricant and chondroprotectant in diarthrodial joints. Specifically, we used denatured collagen (without triple-helix structure), amorphous collagen (native triple helical collagen), and fibrillar collagen (aggregates of native triple helical collagen), to determine the effect of collagen structure and conformation on lubricin binding. We also examined how lubricin interacts with collagen and what effect this binding has on friction and adhesion properties. Our results provide new insights into the molecular interactions of lubricin with collagen.

2. Materials and methods

2.1. Materials

Lubricin was purified from human synovial fluid (Jay et al., 2001), and a series of lubricin solutions (50–400 μg/ml) was prepared from stock solution by dilution with phosphate buffered saline (Gibco, 1 × PBS, 1 mM KH_2PO_4, 155 mM NaCl, 3 mM Na_2HPO_4, pH 7.4). The concentration range was chosen to bracket the physiological value of ~200 μg/ml found in synovial fluid (Elsaid et al., 2005).

Type II collagen (from chicken sternal cartilage, Sigma Aldrich) was received as powder and dissolved in 0.25% acetic acid at 1 mg/ml overnight at 4 °C. For amorphous collagen, the dissolved collagen was diluted with PBS to 100 μg/ml concentration. For denatured collagen, the collagen solution was heated to 60 °C for 15 min and then diluted with PBS. For fibrillar collagen, the dissolved collagen was allowed to self-assemble at physiological pH at room temperature until collagen strands were detectable with AFM.

2.2. Preparation of colloidal probes and substrate surfaces

Gold-coated colloidal probes, functionalized with alkanethiol self-assembled monolayers (SAMs) and various forms of collagen, were fabricated by gluing 10 μm diameter glass microspheres (Duke Scientific, Palo Alto, CA) to the end of AFM cantilevers (V-shaped Si_3N_4 cantilever with 0.58 N/m typical spring constant, Bruker-Veeco™) with a one part photo-curing epoxy (Norland Optical Adhesive #81) (Chang et al., 2008). The probes were then coated with a 5 nm chromium adhesion layer followed by a 45 nm gold layer using an e-beam evaporator (CHA Industries, Fremont, CA).

Type II collagen was chemically attached to gold-coated glass substrates and colloidal probes with glutaraldehyde coupling chemistry (Fig. 1). First, the gold substrates and colloidal probes were cleaned with UV ozone for ~5 min, rinsed with 0.05% SDS, dH_2O, and ethanol, and then incubated in 1 mM NH_2-terminated SAM (cystamine hydrochloride, Sigma Aldrich) in ethanol overnight at room temperature. After incubation, the substrates were sonicated briefly to remove excess thiol (with exception of the colloidal probes) and then rinsed with copious amounts of ethanol and dried with nitrogen. The amine-terminated SAM were activated in 12% glutaric dialdehyde (Sigma Aldrich) for 30 min and then incubated in 100 μg/ml of the collagen solution of interest for over 2 h. Fibrillar collagen substrates were obtained by chemically attaching the fibrils onto the substrate surface, using the procedure described above.

2.3. Characterization of collagen structure

2.3.1. CD

Circular dichroism spectroscopy (CD) was used to monitor the secondary molecular structure of collagen. The collagen solution with a concentration of 0.04 mg/ml in 0.25% acetic acid was measured with a CD spectrophotometer, equipped with a stirred thermoelectric cell holder (Aviv model 202 CD spectrophotometer). CD spectra were acquired over the wavelength range of 190–260 nm at 25 °C before and after heating. The thermal denaturation of collagen was monitored by observing the CD signal at 220 nm over the range of 25–60 °C with a 10 s averaging time and a 2 min equilibration time. The resulting data were baseline corrected by solvent subtraction.

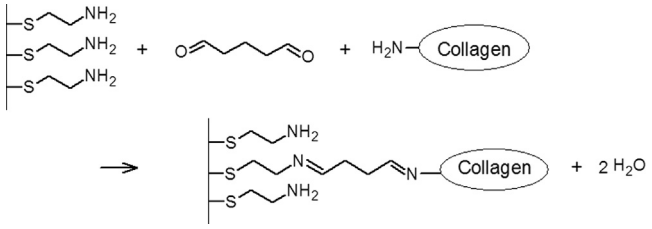

Fig. 1. Chemical attachment of collagen to amine-functionalized surfaces with glutaraldehyde.

2.3.2. XPS

X-ray photoelectron spectroscopy (XPS) (Axis Ultra, Kratos Analytical) was used to verify the surface chemical composition. A monochromatic Al source was used (10 mA, 15 kV) for both the survey and high resolution scans. Survey spectra from 0 to 1200 eV were taken followed by high resolution scans around the C_{1s}, and N_{1s} regions. To avoid degradation of the nonconductive samples, charge neutralization with low energy electrons and a magnetic immersion lens was used. The spectra were shifted by setting the binding energy maximum of C_{1s} at 284.5 eV.

2.3.3. AFM

The substrate surfaces were imaged in Tapping Mode® with a Multimode AFM using a Nanoscope IIIa controller (Bruker-Veeco, Santa Barbara, CA) using an ultrasharp silicon tip (NSC14, MikroMasch, San Jose, CA). Fibrillar collagen formation was verified by AFM imaging, by incubating a freshly cleaved mica surface with ~40 μL collagen fibril solution for ~15 min, followed by rinsing gently with MilliQ water, and drying under a stream of nitrogen. After chemical attachment, the collagen surfaces were imaged again at the lowest applied load.

2.3.4. Ellipsometry

Dry collagen film thicknesses were measured on a spectroscopic ellipsometer (M-88 Model 450, J. A. Woollam Co., Lincoln, NE) at 65°, 70°, and 75°. For each substrate, ellipsometric data was collected on the gold surface before and after collagen deposition. The collagen film thickness was obtained by fitting the ellipsometric data with a Cauchy dispersion model on top of the bare gold layer (Ma et al., 2004). The film thickness of each sample was measured at three different locations and is reported as an average value.

2.3.5. Contact angle

A contact angle goniometer (Model 100, Rame-Hart Instrument Co., NJ) was used to measure the contact angle on the collagen substrates. Static contact angles were taken in ambient condition at room temperature with Milli-Q grade water (18.2 MΩcm) on at least 4 different locations on the same sample and reported as an average value.

2.4. Adsorption measurements

Lubricin adsorption onto collagen surfaces was measured using surface plasmon resonance (SPR Biacore X instrument, Uppsala, Sweden) (Chang et al., 2008). Prior to any measurement, the substrate surfaces were first equilibrated with PBS (pH ~7.4), injected at a flow rate of 5 μL/min at 25 °C. The adsorption of lubricin on collagen surfaces was achieved by sequential injection of 15 μl solution from 25 to 400 μg/ml concentrations. After each lubricin injection, a continuous flow of PBS was used to rinse away any loosely adsorbed molecules. The change in the response unit (RU) before and after each injection is proportional to the number of molecules adsorbed on surface. As a reference, for globular proteins, 1 ΔRU is equivalent to a surface coverage of approximately 1 pg/mm^2.

2.5. Force measurement

A MFP-3D AFM (Asylum Research, Santa Barbara, CA) was used to measure the friction and normal interaction forces. The cantilever normal spring constant (k_n, nN/nm) was determined prior to any measurements from the power spectral density of the thermal noise fluctuations in air (Hutter and Bechhoefer, 1993) by fitting the first resonance peak to equations for a simple harmonic oscillator (Walters et al., 1996). The normal photodiode sensitivity (S, nm/V), was determined from the constant compliance regime upon approach against a hard substrate. The