STRUCTURAL AND TRIBOLOGICAL ANALYSIS OF HEALTHY AND PATHOLOGICAL SYNOVIAL FLUIDS

Constantin Ionut MATEI1,2), Ana-Maria TRUNFIO-SFARGHIU1), Bogdan MUNTEANU1), Marie-Genevieve BLANCHIN2), Pierre MIOSSEC3), Pierre BOULANGER4), Saw-See HONG4), Y. BERTHIE1)

1) LaMCoS UMR5259, INSA-Lyon, CNRS, F-69621, University of Lyon, Villeurbanne Cedex, FRANCE
2) LPMCN, UMR5586, University Claude Bernard Lyon1, University of Lyon, Villeurbanne Cedex, FRANCE
3) Research Unit Immunogenomics and inflammation, Hospital Edouard Herriot, University of Lyon, FRANCE
4) Laboratory of Retrovirus and Comparative Pathology, UMR754 INRA-UCBL-EPHE, University of Lyon, Lyon, FRANCE

constantin-ionut.matei@insa-lyon.fr

ABSTRACT

Recent studies have shown the existence of two types of discontinuous lipid structures in the synovial fluid lubricant film: (i) the lipid multilayers which form the synovial fluid-cartilage interface [Purbach, Clin.Orthop.Relat.Res.2002], and (ii) lipid vesicles present within lubricant films [Watanabe, Med.Electron.Microsc.2000]. The size of these lipid vesicles, compared to the thickness of the lubricant film estimated by hydrodynamics lubrication models [Jin and Dowson, ProcI.MEJ.J.Eng.Tribol.2005], is the subject of a controversy. The aim of this study was to analyze the role of discontinuous lipid structures in the lubrication mechanism in healthy and pathological joints.

Keywords: Bio lubrication, transmission electron microscopy, synovial joints, discontinuous lipid structures

1. INTRODUCTION

Synovial joints have been studied since the '50 because of their remarkable properties allowing them to sustain various types of movement and also because of their exceptional lifetime of up to 80 years. These studies aimed to improve the understanding of the powerful mechanisms of joint lubrication, in particular the tribological behavior and the interactions between cartilage and synovial fluid (SF). The first hypotheses over the synovial joint’s extraordinary reliability have proposed that biolubrication mechanisms consist of full-fluid film lubrication [1]. This theory considered the synovial fluid as a continuous full-fluid lubricating film separating the cartilages, both friction and wear being reduced because the two surfaces loaded against one another are completely separated by a thin film of lubricant of ~ 0.5 μm.

Recent biological studies showed the presence of discontinuities in the lubricating film, for example vesicles of few μm were identified in rat synovial fluid samples [2]. These discontinuities are larger than the lubricant film’s thickness estimated with hydrodynamic models [3].

The origin of these discontinuities is related to the synovial fluid’s molecular composition. Physicochemical studies have shown that when associated with hyaluronic acid (HA), lipids form multilayered vesicular and tube-like structures filled with HA [4]. Furthermore, it has been shown that the inclusion of lipid vesicles in HA or the inclusion of HA in lipid vesicles modifies the rheological behavior of HA aqueous solutions [5]. Moreover SF proteins (albumin, γ-globulin...) can interact with HA and hence change the lubricant film’s rheology [6]. They can also interact with the rubbing surfaces changing the lubrication mechanism [7].
In this context, the aim of this study was to compare the evolution of structural and tribological properties of the synovial fluids taken from healthy or pathological joints with biomimetic fluids prepared having various structures, in order to understand the possible mechanism. Thus, the synovial fluid samples have been studied by coupling:
- Transmission Electron Microscopy (TEM) analyses, in order to characterize the vesicular structures of our samples
- Tribological analysis, to understand the evolution during the friction of molecular structures in healthy and pathological case.

2. METHODS

2.1. Sampled Synovial Fluids

The healthy synovial fluid samples were collected from dog subjects [male, 10 kg, race Beagle] in full compliance with the ENVL (Ecole Nationale Vétérinaire de Lyon) ethical committee guidelines for animal protection and in accordance with the legislation of the European Community.

The pathological synovial fluids samples were collected from human patients with different joint diseases from Edouard Herriot Hospital in Lyon (France).

2.1 Reconstituted Biomimetic Synovial Fluids

The biomimetic synovial fluids was reproduced respecting the molecular composition of healthy synovial fluid [8] from: 3mg/ml fluid phase phospholipids (DOPC 770375 Avanti Polar Lipids), 3mg/ml Hyaluronic Acid (H7630, Sigma-Aldrich), 18mg/ml bovine serum albumin (A3059, Sigma-Aldrich) and 2mg/ml γ-Globulin (G4386). We first prepared the following two solutions:
- a solution of 3 g/l lipids with 1% (wt%) fluorescent lipids (NBD-PC, 810130 Avanti Polar Lipids) in a solvent composed of 90% chloroform and 10% ethanol in volume,
- a solution of 3 g/l hyaluronic acid and 18 g/l bovine serum albumin and 2 g/l γ-Globulin in PBS buffer pH=7.4, this solution will further be referred to as “glycoprotein gel”.

An appropriate aliquot of the solvent lipid solution was poured in a glass tube and the solvent was evaporated under a stream of nitrogen. The resulting lipid film was then kept under high vacuum overnight to ensure the absence of organic solvent traces.

This lipid film was resuspended with different protocols in order to obtain two types of biomimetic synovial fluid structures. First, the glycoprotein gel was added over the lipid dried film covering the glass tube and maintained for 48 h at 37°C on a magnetic stirrer. This first protocol was used in order to obtain a synovial fluid structure composed of lipid vesicles containing glycoprotein gel and will be referred as “included glycoprotein gel” (gel in), simulating the healthy synovial fluid.

The second protocol was used in order to obtain a synovial fluid structure of glycoprotein gel containing lipid vesicles. This structure will be referred as “non-included glycoprotein gel” (gel out), simulating the pathological synovial fluid. For that, multilamellar vesicles (MLVs) were obtained by hydrating the dry lipid film in 15 mM Tris buffer pH 7.4 at room temperature to a final concentration of 20 mg/ml. MLVs were vortexed for 10 min, frozen for 5 min in liquid nitrogen and then thawed for 10 min in a water bath (37°C), the whole procedure being repeated six times and in between each cycle MLVs were vortexed for 1 min. The resulted MLVs solution was vortexed for 10 min with the glycoprotein gel.

2.3. Transmission Electron Microscopy (TEM)

All the samples were prepared for TEM using negative staining as specimen contrast protocol [9]. About 10 μl of synovial fluid was placed for 5 minutes on 3 mm wide electron microscopy copper grids previously covered by carbon (Agar Scientific, S160 Carbon Film 200 Mesh Cu). For contrasting the lipid membranes the synovial liquid incubated on the grid was stained with 2% phosphotungstic acid and the excess drained using filter paper. After air drying, the specimens were observed by TEM in bright field (BF) image mode, using a TOPCON 02B microscope operated at 120 kV. An example of such a TEM BF image from healthy synovial fluid is presented in Fig. 1.

2.4. Tribological Measurements

A homemade biotribometer (Fig. 2) was used in order to reproduce the realistic operation conditions for a synovial joint [10].
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Fig. 1. Example of TEM BF image showing the structure of synovial fluid on carbon coated grid

Fig. 2. Home-made bio-tribological device

This apparatus enables in situ fluorescence contact visualization and measurement of the friction coefficient between a hydrophilic soft lens HEMA (Hydroxy Ethyl Methacrylate) and a flat borosilicate glass plate, both covered with a lipid bilayer (DLPC 770335, Avanti Polar Lipids) and immerged in different types of synovial fluids. The lipid bilayers on HEMA lens and glass plate were deposited using vesicle burst technique or Langmuir Blodgett technique [11]. Focusing was performed in white light on the projection on the octagonal contour’s surface of the microscope’s field diaphragm because in white light no details, excepting contact’s and HEMA lens’ border, are visible. This focusing was then kept when changing to blue light to view the fluorescent elements of this interface [12].

The HEMA lens was chosen because, when submerged in saline buffered solution (150mM NaCl, pH7.4), it contains 25% water (wt%) and its mechanical properties are similar to those of articular cartilage [13].

For the present study a 0.3 MPa contact pressure (the mean pressure during normal walk) and a relative velocity 0.5 mm/s (used in order to eliminate the influence of any hydrodynamic effects) were used. With soft hydrogels like HEMA, we could not test our device for normal pressures larger than 1 MPa because the contact area becomes too large and unstable during sliding. However, we did not notice any tribological change at 1 MPa. Concerning the sliding velocity, we experimentally tested the range 0.1-1 mm/s and we did not notice any speed dependence of the friction coefficient indicating that we are in the boundary lubrication regime.

4. RESULTS

The TEM results showed structural similarities between sampled and biomimetic synovial fluids: the presence of multilamellar vesicles for the healthy case and when the glycoprotein gel is included (Fig. 3 A, B) and unilamellar vesicles for the pathological case and when the glycoprotein gel non-included (Fig. 3 C, D).

Fig. 3. TEM bright field imaging for sampled (A - healthy, C - pathological) and biomimetic (B - included glycoprotein gel, D - non-included glycoprotein gel) synovial fluids
Our tribological data (Fig.4), suggested that, in healthy synovial fluids, the velocity accommodation mechanism was provided by both the lipid multilayer structures at the interfaces and the lipid vesicles within the film. This resulted in a low friction coefficient (0.01). In pathological synovial fluids however, the velocity accommodation was provided by the glycoprotein gel non-included in vesicles, and the interface with lipid structures progressively disappeared. This type of velocity accommodation increased the friction coefficient (0.15-0.2).

Fig. 4. Fluorescence visualization, friction coefficient measurements and velocity accommodation in the molecular assemblies of sampled (A, C) and reconstituted (B, C) synovial fluids

5. CONCLUSIONS

This study showed that the presence of the lipid vesicles with the gel glycoprotein included, maintains a low and stable friction coefficient in healthy cases, while the presence of glycoprotein gel non-included in vesicles increases the friction coefficient in pathological cases by nearly one order of magnitude.

REFERENCES