Liposomes Act as Effective Biolubricants for Friction Reduction in Human Synovial Joints

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Received July 8, 2009. Revised Manuscript Received November 15, 2009

Phospholipids (PL) form the matrix of biological membranes and of the lipoprotein envelope monolayer, and are responsible for many of the unique physicochemical, biochemical, and biological properties of these supramolecular bioassemblies. It was suggested that phospholipids present in the synovial fluid (SF) and on the surface of articular cartilage have major involvement in the low friction of cartilage, which is essential for proper mobility of synovial joints. In pathologies, such as impaired lubrification (leading to common joint disorders such as osteoarthritis), the level of phospholipids in the SF is reduced. Using a human-sourced cartilage-on-cartilage setup, we studied to what extent and how phospholipids act as highly effective cartilage lubrificants. We found that large multimamellar vesicles (MLVs), > 800 nm in diameter, composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or of a mixture of DMPC and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) are superior lubrificants in comparison to MLVs composed of other phosphatidyl cholines. Introducing cholesterol into liposomes resulted in less effective lubricants. DMPC-MLV was also superior to small unilamellar vesicles (SUV), < 100 nm in diameter, composed of DMPC. MLVs are superior to SUV due to MLV retention at and near (<200 μm) the cartilage surface, while SUV penetrate deeper into the cartilage (450–730 μm). Superiority of specific PL compositions is explained by the thermotropic behavior (including compressibility) of the lipid bilayer. Correlating physicochemical properties of the MLVs with the friction results suggests that MLVs having lipid bilayers in the liquid-disordered phase and having a solid-ordered to liquid-disordered phase transition temperature slightly below physiological temperature are optimal for lubrication. High phospholipid headgroup hydration, high compressibility, and softness are the common denominators of all efficient PL compositions. The high efficiency of DMPC-MLV and DMPC/DPPC-MLV as cartilage lubricants combined with their resistance to degradation at 37 °C supports further evaluation of these MLVs for treatment of joint impairments related to poor lubrication. This work also demonstrates the relevance of basic physicochemical properties of phospholipids to their activities in biological systems.

Introduction

Phospholipids (PL), which form the matrix of biological membranes’ bilayer and of lipoproteins’ monolayer envelope, are responsible for many of the unique physicochemical, biochemical, and biological properties of these supramolecular bioassemblies.1,2 It has been suggested that PL present in the SF facilitate low friction in articulating cartilage, a property which is essential for proper mobility of synovial joints.3–5 Joint dysfunctions affect a very large portion of the population. Sufficient lubrification is a prerequisite for proper joint mobility, which is crucial for prevention and amelioration of degradative changes of the joint.6 A common joint dysfunction is osteoarthritis (OA), with a prevalence exceeding 20 million in the United States alone.7 The etiology of OA is multifactorial, including inflammatory, metabolic, and mechanical causes.6,8 Current treatment of OA focuses on joint mobilization, which is enabled by avoiding overloading as well as control of pain and inflammation using medications administered systemically or intra-articularly.9

Articular cartilage forms a smooth, tough, elastic, and flexible surface that facilitates bone movement. The synovial space is filled with the viscous synovial fluid (SF) containing hyaluronic acid (HA) and the glycoprotein lubricin.10–12 HA is a polymer of D-glucuronic acid and D-N-acetylgalactosamine, which degrades under inflammatory conditions such as in OA.13,14 Lubricin is composed of ∼44% proteins, ∼45% carbohydrates, and ∼11% surface active phospholipids (SAPL).10–12 of which ∼41% are phosphatidylcholines (PCs), ∼27% phosphatidylethanolamines (PEs), and ∼32% sphingomyelins.3,4,15
Boundary lubrication, in which layers of lubricant molecules separate opposing surfaces, occurs under loading of articular joints. Several different substances have been proposed as the native boundary lubricants of articular cartilage. In the past, HA was considered to be a major cartilage lubricant and was approved by the FDA for treatment of OA of the knee; however, more recent studies demonstrate that HA is not an efficacious lubricant, and an in-depth meta-analysis indicates that intra-articular administered HA has a small effect when compared with an intra-articular placebo. Other reports ascribe the lubricating properties of SF to lubricin. Pickard et al. and Schwarz and Hills demonstrated that SAPL of lubricin facilitate joint lubrication in articular cartilage. Hills and co-workers demonstrated that OA joints have a SAPL deficiency and that injection of the SAPL 1,2-dipalmitoylphosphatidylcholine (DPPC) in propylene glycol into human OA joints resulted in mobility improvement lasting up to 14 weeks without major side effects. Gardner et al. demonstrated the presence of spherical deposits on the surface of dog articular cartilage, resembling lipid components. A later report by Watanabe et al. observed globular lipidic vesicles localized in the thick upper surface layer of healthy cartilage, which are assumed to play a major role in lubrication. Kawano et al. and Forsey et al. using animal models, have shown that DPPC liposomes combined with high molecular weight HA (∼2000 kDa) had better lubricating ability than HA alone. Recently, Klein and co-workers summarized various issues of joint lubrication at the molecular level. They point to the potential role of highly hydrated brush-like charged macromolecules at the surface of cartilage as major contributors to cartilage lubrication.

### Materials and Methods

**Lipids.** Table 1 describes the lipids (>98% pure) used in this study, their source, bilayer phase transition temperature ($T_m$), and bilayer physical phase at 37 °C.

<table>
<thead>
<tr>
<th>lipids</th>
<th>chemical name (source)</th>
<th>MW</th>
<th>acyl chains, (number of C: unsaturated bonds)</th>
<th>phase transition temperature ($T_m$), °C</th>
<th>physical phase at 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBPC</td>
<td>1,2-dioleoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL)</td>
<td>902.4</td>
<td>22:0, 22:0</td>
<td>74</td>
<td>SO</td>
</tr>
<tr>
<td>HSPC</td>
<td>hydrogenated soybean phosphatidylcholine (Lipoid, Ludwigshafen, Germany)</td>
<td>762.1</td>
<td>86% 18:0, 13% 16:0, 1% others</td>
<td>52.5</td>
<td>SO</td>
</tr>
<tr>
<td>DPPC</td>
<td>1,2-dipalmitoyl-sn-glycero-3-phosphocholine (Avanti or Lipoid)</td>
<td>734.1</td>
<td>16:0, 16:0</td>
<td>41.4</td>
<td>SO</td>
</tr>
<tr>
<td>DMPC</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphocholine (Lipoid or Avanti)</td>
<td>677.9</td>
<td>14:0, 14:0</td>
<td>23.2</td>
<td>LD</td>
</tr>
<tr>
<td>DOPC</td>
<td>1,2-dioleoyl-sn-glycero-3-phosphocholine (Lipoid or Avanti)</td>
<td>786.2</td>
<td>18:1, 18:1</td>
<td>21</td>
<td>LD</td>
</tr>
<tr>
<td>mPEG-DSPC</td>
<td>R. W. Fisher poly(ethylene glycol) diesteroyl phosphatidylethanolamine (Genzyme, Liestal, Switzerland)</td>
<td>2774</td>
<td>18:0, 18:0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>(3β)-cholesterol-5-en-3-ol (Avanti or Sigma)</td>
<td>386.7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*a All PCs described form a lamellar phase and are vesicle-forming lipids. L/D, liquid disordered phase; SO, solid ordered phase; NA, not applicable. $T_m$ is the temperature at which the maximal change in heat capacity occurs during the SO-to-LD phase transition. $T_m$ for DBPC, DPPC, DMPC, and DOPC are from Marsh et al., and for HSPC from Khazanov et al. mPEG-DSPC is a micelle-forming amphiphile.*

**Bicarbonate buffer.** A commercial bicarbonate buffer solution (95% H2O, 4.5% CO2), was prepared using a WaterPro PS HPLC/Ultrafilter Hybrid system (Labconco, Kansas City, MO).

**Other Reagents.** All other reagents used were of analytical grade or better.

**Liposomes.** Multilamellar vesicles (MLV) composed of pure PCs (DMPC, DPPC, HSPC, DBPC, or DOPC or mixtures of DMPC/cholesterol (2:1 mol/mol), and DMPC/mPEG-DSPC (95:5 mol/mol) or DMPC/DPPC (0:6:1.0 mol/mol), were prepared by dissolving the desired lipids in terti-butanol, followed by lyophilization to form a dry “cake”. This was hydrated in low ionic strength (5 mM) histidine buffer (HB) pH 6.7, at temperatures of at least 5 °C above the lipid $T_m$. When needed, MLV...
were downsized to form small unilamellar vesicles (SUV, < 100 nm) by stepwise extrusion through polycarbonate membranes (GE-Osmontics, Minnetonka, MN), starting with a 400 nm and ending with a 50 nm pore-size membrane, using a 10 mL extrusion system (Northern Lipids, Vancouver, Canada) heated at least 5 °C above the \( T_m \).32

**Liposome Characterization.** Vesicles were characterized for (i) phospholipid (PL) concentration using the modified Bartlett assay33 and (ii) size distribution, for vesicles under 1 μm by dynamic light scattering using an ALV-NIBS/HPPS (Langen, Germany) particle sizer at a scattering angle of 173°, and for those above 400 nm, by light diffraction using a Beckman Coulter LS Particle Size Analyzer 13–320 (Fullerton, CA) equipped with polarization intensity differential scattering (PIDS) to provide a detection range up to 2000 μm; (iii) partial specific adiabatic compressibility, by measuring the density of the dispersion (using a DMA 5000 density meter, Anton Paar, Graz, Austria) and the velocity of a 5 MHz ultrasonic wave traveling through it (using a UCC-12 ultrasonic velocimeter, NDT Instruments, Jerusalem, Israel), and then calculating the compressibility as described by Garbuzenko et al.34

Bulk viscosity of liposomal dispersions was measured at 25 °C using a Cannon Manning Semi Microviscometer 100-B8 (Cannon Instrument Company, State College, PA).

**Cartilage.** This study was approved by, and conducted according to, the Technion (Haifa, Israel) Research Ethics Committee and with donors’ consent.

Articular cartilage from healthy donors (aged 65 to 86 years) was obtained from total hip replacements, due to fractures of the hip joint, and was used for friction tests. Similarly-obtained OA specimens were used as a control for measurement of the total phospholipid concentration in cartilage. OA specimens were not subjected to friction tests and were not graded. For the reliability and accuracy of the data measured, all friction tests were performed using healthy and not OA cartilage, due to inconsistency and possible multiple grades present within OA specimens, which might result in large error with no sound conclusions.

Plugs of cartilage, down to the bone (approximately 1.5–2 mm thick), preferably from the same region of the joint (femoral head), were removed from the bone using a cork-borer and a scalpel35 and trimmed on the bone side using a 1320 Leica freezing microtome (Wetzlar, Germany), in order to allow good attachment to the holders. Up to 12 pairs of cartilage discs, of 4 and 8 mm diameter for each pair, were harvested from each of the femoral heads and kept at \(-20^\circ\)C until used. The cartilage discs were then glued to holders on their trimmed side (close to the bone side) prior to measurements, using a cyanoacrylate-based glue. Friction tests were carried out on the untrimmed, i.e., the exposed, side of the cartilage samples.

**Friction Testing.** Liposomes covering a wide range of sizes and concentrations were screened as potential lubricants to reduce friction between two discs of human cartilage at 24 and 37 °C. Friction testing was carried out using a cartilage-on-cartilage setup (Figure 1a), in which two discs of healthy human cartilage are immersed in HB, or in saline (0.9% w/v; pH 5.0; Teva Medical, Israel), or in inflated synovial fluid (SF, obtained from knee surgeries of healthy individuals), or in 10 mg/mL HA in HB, or in liposomal dispersions. Synovial fluids were obtained with donors’ consent. Both cartilage discs were not entirely confined, and a certain portion of the cartilage in both discs was extended beyond its holder in order to allow good contact with its opposing cartilage disc (Figure 1a).

These discs were subjected to relative sliding over a wide range of loads (1 to 30 N), equivalent to physiological pressures in joints (0.08 to 2.4 MPa), at sliding velocities of 0.5 to 2 mm/s and dwell times of 5–300 s, to simulate a range of physiological movements.

A schematic representation of the friction measurement setup is shown in Figure 1a. The normal load, \( N \), is provided by dead weights, and the friction force, \( F \), is measured continuously by a load cell with a strain gauge measuring system (Wagezelle Z8, Hottinger Baldwin Messtechnik, Darmstadt, Germany). The desired sliding speed and distance were preset and controlled by computer using LabVIEW software, which also calculates the instantaneous friction coefficient \( \mu = F/N \) and plots it against time. Figure 1b presents a typical plot from a friction test at a sliding velocity of 1 mm/s of the upper, 4-mm-diameter cartilage disc over the lower, 8-mm-diameter disc. As can be seen, the

![Figure 1. Cartilage-on-cartilage friction measurement setup.](image-url)

Friction measurements were carried out using a cartilage-on-cartilage test setup drawn schematically (a). Two discs of cartilage were glued to appropriate holders to obtain good conformal contact of their rubbing surfaces, as in natural joints. An upper cartilage disc (4 mm diam) is loaded with a force \( N \) against a lower cartilage disc (8 mm diam). The two discs are immersed in a bath containing the lubricant, and the lower cartilage specimen is pulled horizontally at a controlled velocity while the friction force, \( F \), between the discs is continuously measured and the friction coefficient is calculated and plotted. A typical plot from a test at a sliding velocity of 1 mm/s is shown in (b). The static friction coefficient is obtained from the maximum value, indicated by point (1). The dynamic friction coefficient is the average of the values measured over the sliding time.

OA patients, or in healthy synovial fluid (SF, obtained from knee surgeries of healthy individuals), or in 10 mg/mL HA in HB, or in liposomal dispersions. Synovial fluids were obtained with donors’ consent. Both cartilage discs were not entirely confined, and a certain portion of the cartilage in both discs was extended beyond its holder in order to allow good contact with its opposing cartilage disc (Figure 1a).

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friction coefficient increases during a short presliding period of ∼1 s, to reach a maximum, and then decreases gradually while sliding takes place over the remaining time of the test. The static friction coefficient is obtained from the maximum value indicated in Figure 1b by point (1). The dynamic friction coefficient is the average of the values measured during the sliding period.

In order to distinguish among the effects of the different parameters under investigation, each specimen pair was tested with only one lubricating fluid. Tests were performed in increasing normal load order. The same practice was also used for studying the effect of dwell time, temperature, and sliding velocity.

Dwell times in the range from 5 s to 5 min were tested to mimic natural processes in synovial joints. The recovery time, i.e., the time without any load between successive repetitions, was at least as long as the corresponding dwell time to allow fluid intake into the cartilage to occur.

For each tested pair of cartilage discs, only one parameter was changed while all other parameters were kept constant. One dedicated pair was used for each friction test. All reported friction coefficients are the means of data obtained from at least 3 different pairs with at least 10 repetitions per specimen pair.

**PL Extraction and Quantification.** Total PL was extracted from cartilage specimens, preferably taken from the same loading area, before (control) or after conducting friction tests using the Bligh and Dyer extraction procedure. In this case, the cartilage discs were incubated in a chloroform–methanol solution (1:1 v/v) for 1 h at 37 °C. Water was added to a final chloroform–methanol–water ratio of 1:1:1, the solution was vortexed for 1 min and then centrifuged to form two phases. The chloroform-rich lower phase, containing >99% of PL, was dried under vacuum (Concentrator 5301, Eppendorf), and total PL was quantified by organic phosphorus determination using the modified Bartlett procedure. In addition, PC concentration was quantified as a function of cartilage depth. For this, cartilage discs were sectioned by microtome into slices 20 or 50 μm thick, from the cartilage surface inward, parallel to the face of the cartilage. PL of each of the slices were extracted and concentrated as described above. Then, PL were redissolved in a small volume of chloroform–methanol (2:1 v/v) and loaded onto low-phosphorus silica gel TLC glass plates (Uniplate – Silica Gel G, Analtech, Newark, DE). A chloroform–methanol–water (65:25:4 v/v/v) solvent system was used for chromatographic separation on TLC plates. Commercial markers of sphingomyelin, PC, lyso PC, and PE were also loaded on the TLC plates for spot identification. Lipid spots were detected by spraying the dried TLC plates with a UV-detectable primulin (Sigma) solution (1 mL of 0.1% w/v primulin in water, added to 100 mL acetone–water, 4:1 v/v). The PC spot of each slice was scraped from the TLC plate, and its organic phosphorus content was quantified by the modified Bartlett procedure.

**Physical and Chemical Integrity of MLV.** The physical and chemical integrity of DMPC-MLV were assessed after incubation in SF, or ISF, or HB for ∼40 h at 37 °C. Every 8 h a sample was collected, MLV size distribution was assessed using a Beckman Coulter (as above), and phase contrast optical microscopy (using an Eclipse TE2000-E inverted microscope equipped with a Plan Apo 60× lens, both from Nikon). PL were extracted by the Bligh and Dyer procedure, and the chemical integrity was determined by TLC, using three different mobile systems (chloroform–methanol–water, 65:25:4 v/v/v; or chloroform–acetonitrile–methanol–acetic acid–water, 6:8:2:2:1; or chloroform–methanol–ammonia–water, 65:25:0.4:4). Similarly, the chemical integrity of DMPC-MLV was evaluated by somewhat different exhaustive friction tests (450,000 reciprocating cycles under a load of 60 N during 7 h at 37 °C).

**Results**

It is worth noting that friction coefficients for normal joints were reported to be in the range between 0.0005 and 0.04. This large range is due to the fact that friction coefficient is a relative value, which depends on many system properties including viscosity, sliding velocity, and normal load, as demonstrated by the classic Stribeck Curve. (The Stribeck Curve is a plot which relates between the friction coefficient and viscosity, speed and load. It presents the three regimes of lubrication: boundary, mixed, and hydrodynamic lubrication.)

**Effect of Load on Friction Coefficients of Cartilage Lubricated with PC-MLV of Various Compositions.** MLV composed of PCs (DMPC, DPPC, HSPC, or DOPC, all at 40 ± 5 mM, and DBPC at 7.5 mM (due to DBPC precipitation beyond this concentration)) varying in their acyl chains, and therefore in their T_m (Table 1), all dispersed in HB, were screened for their cartilage-lubricating abilities under different loads, representing a wide range of physiological postures. Generally, as load increased, both static and dynamic friction coefficients decreased (Figure 2a,b). Under all loads, cartilage lubricated by DMPC-MLV resulted in the lowest friction coefficients.

**Effect of DMPC-MLV Concentration, Viscosity, Dwell Time, and Sliding Velocity on Friction.** For further comparisons between different PC compositions, the effect of lipid concentration on cartilage friction coefficients was measured using the most effective lubricant found earlier, DMPC-MLV, under loads of 10 and 30 N and sliding velocity of 1 mm/s. Friction coefficients decreased to a minimum at a concentration of ∼140 mM, after which they increased, reaching a plateau at ∼330 mM (Figure 2c). The decrease in friction up to a concentration of ∼140 mM is explained by the increase in PL (lubricant) concentration at low bulk viscosity (<12 cP up to a DMPC concentration of ∼150 mM) of the dispersion, while at higher concentrations, bulk viscosity increases substantially (30 cP at 240 mM, and 90 cP at 380 mM), thus increasing friction.

The choice of using a sliding velocity value of 1 mm/s throughout this study followed our investigation on the effect of velocity on friction under different loads. It was found that, as sliding velocity increases, dynamic friction coefficients decrease. The dynamic friction coefficients of cartilage lubricated with DMPC-MLV (140 mM, at 37 °C) were 0.0534 ± 0.0114, 0.0240 ± 0.0025, and 0.0231 ± 0.0058 at velocities of 0.5, 1, and 2 mm/s, respectively, and of cartilage lubricated with saline were 0.0756 ± 0.0089, 0.0647 ± 0.0094, and 0.0351 ± 0.0041, respectively—all under a load of 30 N. A similar pattern was found under a load of 10 N.

It was found that an increase in dwell time resulted in a significant increase in friction coefficient (Figure 2d). For the dwell time, a typical value of 5 s was used throughout this study.

**Effect of Temperature on the Lubricating Efficiency of Various Media and MLV PC Compositions.** In a separate experiment, the static and dynamic friction coefficients of cartilage lubricated with different MLV PC compositions (DMPC, DPPC, HSPC, or DOPC, all at a concentration of 140 mM in HB) were reported to be in the range between 0.0005 and 0.04. This large range is due to the fact that friction coefficient is a relative value, which depends on many system properties including viscosity, sliding velocity, and normal load, as demonstrated by the classic Stribeck Curve. (The Stribeck Curve is a plot which relates between the friction coefficient and viscosity, speed and load. It presents the three regimes of lubrication: boundary, mixed, and hydrodynamic lubrication.)


Langmuir 2010, 26(2), 1107–1116
were compared at 24 and 37 °C under a load of 30 N and sliding velocity of 1 mm/s. These PCs differ in their acyl chains, which determine the basic characteristics of the vesicles, especially their Tm and physical phase (Table 1). For all MLV-PC compositions the friction coefficients are lower at 37 °C in comparison to 24 °C (Table 2). The lowest static and dynamic friction coefficients at both temperatures were achieved using DMPC-MLV.

The effect of temperature on friction can be described using an Arrhenius plot, relating the natural log of the static friction coefficient and the reciprocal of the absolute (Kelvin) temperature. The highest energy of activation calculated from the

Table 2. Static and Dynamic Friction Coefficients of Cartilage Lubricated with Multilamellar Vesicles (MLV) Composed of Different Phosphatidylcholines (PCs) at 140 mM in HB, or with Media Alone, at 24 and 37 °C, under a Load of 30 N, Sliding Velocity of 1 mm/s, and Dwell time of 5 s

![Figure 2](image-url)

**Figure 2.** (a,b) Effect of load on static and dynamic friction coefficients. Generally, as the normal load increases, friction coefficients decrease. Phospholipid concentration is 40 ± 5 mM, except for DBPC which is 7.5 mM due to lipid precipitation beyond this concentration, sliding velocity 1 mm/s, dwell time 5 s at 24 °C. (c) Static and dynamic friction coefficients at different DMPC-MLV concentrations under loads of 10 and 30 N. Friction coefficients decrease to a minimum at ~140 mM, after which they increase, reaching a plateau at a concentration of ~330 mM. Sliding velocity 1 mm/s, dwell time 5 s at 37 °C. (d) Effect of dwell time on friction coefficients. As dwell time (i.e., the time between applying load and initiating motion) increases, friction increases. This may be caused by the effect of creep as dwell time increases. Experiments were conducted under a load of 30 N at 37 °C, sliding velocity of 1 mm/s, and DMPC-MLV concentration of 140 mM.

<table>
<thead>
<tr>
<th>Composition of MLV (in HB)</th>
<th>Static Friction Coefficient at 24 °C</th>
<th>Dynamic Friction Coefficient at 24 °C</th>
<th>Static Friction Coefficient at 37 °C</th>
<th>Dynamic Friction Coefficient at 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DMPC</strong></td>
<td>0.038 (±0.007)</td>
<td>0.024 (±0.009)</td>
<td>0.079 (±0.006)</td>
<td>0.011 (±0.005)</td>
</tr>
<tr>
<td><strong>DPPC</strong></td>
<td>0.052 (±0.008)</td>
<td>0.034 (±0.011)</td>
<td>0.028 (±0.002)</td>
<td>0.022 (±0.002)</td>
</tr>
<tr>
<td><strong>HSPC</strong></td>
<td>0.056 (±0.009)</td>
<td>0.039 (±0.012)</td>
<td>0.044 (±0.006)</td>
<td>0.041 (±0.005)</td>
</tr>
<tr>
<td><strong>DOPC</strong></td>
<td>0.059 (±0.010)</td>
<td>0.040 (±0.009)</td>
<td>0.019 (±0.006)</td>
<td>0.014 (±0.006)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lubricating Medium (with no liposomes)</th>
<th>Static Friction Coefficient at 24 °C</th>
<th>Dynamic Friction Coefficient at 24 °C</th>
<th>Static Friction Coefficient at 37 °C</th>
<th>Dynamic Friction Coefficient at 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histidine Buffer (HB)</strong></td>
<td>0.067 (±0.015)</td>
<td>0.045 (±0.017)</td>
<td>0.053 (±0.009)</td>
<td>0.037 (±0.011)</td>
</tr>
<tr>
<td><strong>Saline</strong></td>
<td>0.089 (±0.008)</td>
<td>0.061 (±0.012)</td>
<td>0.080 (±0.016)</td>
<td>0.060 (±0.013)</td>
</tr>
<tr>
<td><strong>Inflamed Synovial Fluid (ISF)</strong></td>
<td>0.10 (±0.010)</td>
<td>0.085 (±0.008)</td>
<td>0.053 (±0.008)</td>
<td>0.046 (±0.013)</td>
</tr>
</tbody>
</table>

*Italicized cells: best performing lubricants. The friction coefficients were determined as described in Methods and the legend of Figure 1a,b. For lipid abbreviations, see Table 1. Each data point is the mean of 10 measurements ± S.D. The effect of temperature on friction can be described using an Arrhenius plot, relating the natural log of the static friction coefficient and the reciprocal of the absolute (Kelvin) temperature. The highest energy of activation calculated from the
Arrhenius curve was obtained for DOPC-MLV (−15.95 kcal/mol), while DMPC-MLV, DPPC/DMPC (1/0.6)-MLV, and ISF had similar moderate slopes (−9.76, −8.72, and −8.94 kcal/mol, respectively). On the other hand, saline, HB, and the high $T_m$ HSPC-MLV were found to have much lower slopes (−1.50, −3.30, and −3.40 kcal/mol, respectively), suggesting that there is a similarity in the mode of action of the efficacious PCs (DMPC-MLV and DPPC/DMPC-MLV) and of synovial fluids on one hand, while on the other hand, the non-efficacious inorganic media and PCs behave similarly.

Testing the lubricating efficacy of media alone showed that static and dynamic friction coefficients obtained using HB were generally lower than those obtained using saline or ISF (Table 2 and Figure 3) at both 24 and 37 °C. Furthermore, MLV PC compositions dispersed in HB were better lubricants than identical SUV PC compositions dispersed in saline (data not shown).

**Effect of Vesicle Size and Lamellarity on Cartilage Friction Coefficients and on PC Distribution along the Cartilage Depth.** The effect of vesicle size and lamellarity on friction was tested using the best-performing PC: DMPC-MLV (>800 nm in diameter) compared to DMPC-SUV (<100 nm in diameter) at 37°C under a load of 30 N. Static and dynamic friction coefficients of cartilage lubricated with DMPC-MLV were lower (0.019 ± 0.006 and 0.011 ± 0.005, respectively) than those of cartilage lubricated with DMPC-SUV (0.045 ± 0.0032 and 0.036 ± 0.0033, respectively); Figure 3. The friction coefficients of DMPC-SUV were only slightly lower than those measured for the dispersing media (HB) alone (0.053 ± 0.009 and 0.037 ± 0.011, respectively). The MLV superiority over SUV was also confirmed for liposomes of other PC compositions (data not shown).

PC concentration as a function of cartilage depth (0−800 μm, in 20 or 50 μm increments) was measured after friction tests on cartilage lubricated with DMPC-MLV or DMPC-SUV (both in HB), compared to HB alone (control). Figure 4 shows that cartilage lubricated with DMPC-MLV demonstrated the highest PC concentration near the cartilage surface, with a peak at a depth of ~80 μm, below which concentration decreased. In cartilage lubricated with DMPC-SUV, the highest PC concentration was found deeper inside the cartilage (~600 μm), and at the cartilage surface, PC concentration was similar to that of the control (HB alone) (Figure 4).

**PL Levels in Lubricated Cartilage Specimens.** Total PL content of cartilage lubricated with DMPC-MLV was higher than that of cartilage lubricated with DMPC-SUV. The following abbreviations were used: SF, healthy synovial fluid; ISF, inflamed synovial fluid; HB, histidine buffer; HA, hyaluronic acid; for liposome/vesicle abbreviations, see Methods and Table 1. Using t-test, a statistically significant difference ($p < 0.008$) was demonstrated between DMPC-MLV and DMPC/DPPC-MLV (0.6:1.0 mol ratio) and all other lubricants, except for the static friction coefficient value obtained for DMPC/mPEG-DSPE-MLV.

**Figure 3.** Static and dynamic friction coefficients obtained using various lubricants and media. All measurements were performed at lipid concentration of 140 mM at 37°C and under a load of 30 N, sliding velocity of 1 mm/s, and dwell time of 5 s. HA was used at a concentration of 10 mg/mL. The following abbreviations were used: SF, healthy synovial fluid; ISF, inflamed synovial fluid; HB, histidine buffer; HA, hyaluronic acid; for liposome/vesicle abbreviations, see Methods and Table 1. Using t-test, a statistically significant difference ($p < 0.008$) was demonstrated between DMPC-MLV and DMPC/DPPC-MLV (0.6:1.0 mol ratio) and all other lubricants, except for the static friction coefficient value obtained for DMPC/mPEG-DSPE-MLV.

**Figure 4.** PC concentration as a function of vertical depth in cartilage. Cartilage specimens were subjected to friction tests in the presence of (■) DMPC-MLV (>800 nm in diameter) in HB, (▲) DMPC-SUV (<100 nm in diameter) in HB, or (×) HB alone. Then, cartilage specimens were sliced into discs and tested for their PC concentration as a function of cartilage depth (see Methods). All measurements were performed at lipid concentration of 140 mM at 37°C and under a load of 30 N, sliding velocity of 1 mm/s, and dwell time of 5 s.
than that of cartilage lubricated with DMPC-SUV, or HB, or saline, as well as of OA cartilage which was not subjected to friction tests (Figure 5). PL content of cartilage lubricated with HB alone was higher than that of cartilage lubricated with saline and similar to cartilage before friction tests. The total PL level of healthy cartilage lubricated with ISF and exposed to friction tests was similar to that of cartilage obtained from OA patients before friction tests. The pattern of the results described in Figure 5 is in good agreement with that describing the level of PC along the depth of cartilage plugs (Figure 4), where the highest PC content was found in cartilage lubricated with DMPC-MLV.

**Effect of Hydration and Compressibility of MLV Bilayer on Friction Coefficient.** In order to better understand the mechanism of lubrication, we studied the effect of DMPC-based MLV enriched with additional lipids that induce changes in the physical phase and hydration level of the bilayers.

Cholesterol, having a packing parameter of ~1.2 (see Discussion), was added to DMPC at a 1:2 mol ratio, to form DMPC/cholesterol-MLV, in which the lipid bilayers are transformed from the LD phase to the liquid-ordered (LO) phase. The static and dynamic friction coefficients of DMPC/cholesterol-MLV (0.040 ± 0.007 and 0.036 ± 0.007, respectively) were higher than those obtained with DMPC-MLV (0.019 ± 0.006 and 0.011 ± 0.005, respectively) (Figure 3).

The lipopolymer mPEG-DSPE, having a packing parameter of ~0.5, introduces to the lipid bilayer a highly hydrated extended steric barrier that surrounds the vesicle. Addition of mPEG-DSPE to DMPC to form DMPC/mPEG-DSPE-MLV (95/5) did not reduce the static and dynamic friction coefficients in comparison to DMPC-MLV (Figure 3).

The adiabatic compressibility, $K$, which is a measure of the lipid bilayer physical phase (SO, LD, or LO) and level of hydration, was assessed for DMPC, DPPC, and HSPC and found to be 50.7, 31.2, and 33.3 × 10^{-6} mL/(g atm) at 37 °C, respectively, and 46.4, 28.0, and 30.3 × 10^{-6} mL/(g atm) at 24 °C, respectively (experimental error of $K$ values is ±0.3 mL/(g atm)). These $K$ values reflect the higher phase transition temperatures, $T_m$, of DPPC and HSPC (41.4 and 52.5 °C, respectively) compared to that of DMPC (23.2 °C). In DMPC/cholesterol liposomes (2:1 mol ratio), compressibility is reduced to 45.5 and 42.2 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively. Introducing 5 mol% mPEG-DSPE into HSPC liposomes raised compressibility to 35.5 and 32.8 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, while in HSPC/cholesterol liposomes (2:1 mol ratio) compressibility is nearly unchanged, with $K$ values of 33.6 and 30.0 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, in agreement with Garbuzenko et al. The adiabatic compressibility, $K$, which is a measure of the lipid bilayer physical phase (SO, LD, or LO) and level of hydration, was assessed for DMPC, DPPC, and HSPC (41.4 and 52.5 °C, respectively) compared to that of DMPC (23.2 °C). In DMPC/cholesterol liposomes (2:1 mol ratio), compressibility is reduced to 45.5 and 42.2 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively. Introducing 5 mol% mPEG-DSPE into HSPC liposomes raised compressibility to 35.5 and 32.8 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, while in HSPC/cholesterol liposomes (2:1 mol ratio) compressibility is nearly unchanged, with $K$ values of 33.6 and 30.0 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, in agreement with Garbuzenko et al. The adiabatic compressibility, $K$, which is a measure of the lipid bilayer physical phase (SO, LD, or LO) and level of hydration, was assessed for DMPC, DPPC, and HSPC (41.4 and 52.5 °C, respectively) compared to that of DMPC (23.2 °C). In DMPC/cholesterol liposomes (2:1 mol ratio), compressibility is reduced to 45.5 and 42.2 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively. Introducing 5 mol% mPEG-DSPE into HSPC liposomes raised compressibility to 35.5 and 32.8 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, while in HSPC/cholesterol liposomes (2:1 mol ratio) compressibility is nearly unchanged, with $K$ values of 33.6 and 30.0 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, in agreement with Garbuzenko et al. The adiabatic compressibility, $K$, which is a measure of the lipid bilayer physical phase (SO, LD, or LO) and level of hydration, was assessed for DMPC, DPPC, and HSPC (41.4 and 52.5 °C, respectively) compared to that of DMPC (23.2 °C). In DMPC/cholesterol liposomes (2:1 mol ratio), compressibility is reduced to 45.5 and 42.2 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively. Introducing 5 mol% mPEG-DSPE into HSPC liposomes raised compressibility to 35.5 and 32.8 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, while in HSPC/cholesterol liposomes (2:1 mol ratio) compressibility is nearly unchanged, with $K$ values of 33.6 and 30.0 × 10^{-6} mL/(g atm) at 37 and 24 °C, respectively, in agreement with Garbuzenko et al.

**Effect of PC-MLV Phase Transition Temperature on Lubrication.** Our screening results (Table 2) suggest that superior cartilage lubrication by PC-MLV occurs when MLV bilayers are at the LD phase. We further hypothesized that, when the PC-MLV bilayers are in the LD phase, with a $T_m$ slightly below physiological temperature, even lower friction than that of

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DMPC-MLV \((T_m = 23.2 \, ^\circ\text{C})\) can be achieved. To test this hypothesis, we included in our study MLV composed of a combination of DMPC:DPPC \((0.6/1.0, \text{mol/mol})\), which has a \(T_m\) of 34 \(^\circ\text{C}\).\(^{32}\) This composition was selected because of the nearly ideal miscibility of DMPC with DPPC.\(^{42}\) The results support the above hypothesis, as at 37 \(^\circ\text{C}\) DMPC/DPPC-MLV was a more effective lubricant than DMPC-MLV (static and dynamic friction coefficients of 0.017 ± 0.002 and 0.008 ± 0.003 compared to 0.019 ± 0.006 and 0.011 ± 0.005, respectively), but less effective at 24 \(^\circ\text{C}\) (static and dynamic friction coefficients of 0.042 ± 0.007 and 0.021 ± 0.004, compared to 0.038 ± 0.007 and 0.024 ± 0.009, respectively).

Physicochemical Integrity of DMPC-MLV Lubricated in the Presence of ISF and SF. Friction coefficients of cartilage lubricated with SF, or ISF, or DMPC-MLV dispersed in ISF were determined. Static and dynamic friction coefficients (at 37 \(^\circ\text{C}\)) of cartilage in the presence SF \((0.048 ± 0.006\) and 0.034 ± 0.005, respectively) were lower than those obtained in the presence of ISF \((0.053 ± 0.005\) and 0.046 ± 0.008, respectively). However, addition of DMPC-MLV \((140 \, \text{mM})\) to ISF reduced friction coefficients substantially to values found in healthy joints\(^{29}\) (0.024 ± 0.003 and 0.014 ± 0.001, respectively; Figure 3).

TLC analysis of lipid extracts of SF and ISF showed similar lipid components of mainly PC and sphingomyelin. However, the total phospholipid-to-total protein ratio in ISF is lower than that of SF \((8.23 ± 0.93 \, \text{nmol phospholipid per milligram total protein}, \, \text{respectively})\), while the osmolarity of SF \((8.24 \, \text{mOsmol/kg})\), which is in the SO phase at 37 \(^\circ\text{C}\), DMPC/DPPC-MLV (in the SO phase) was a better lubricant than DMPC-MLV (both in the LD phase); however, at 24 \(^\circ\text{C}\), DMPC/DPPC-MLV (in the SO phase) was a less effective lubricant than DMPC-MLV (in the LD phase). DMPC-MLV (in the SO phase at 24 and 37 \(^\circ\text{C}\)) was the least effective lubricant. This suggests that working conditions, being slightly above the SO-to-LD \(T_m\), facilitate highly effective lubrication and may also explain the superiority of DMPC-MLV over DOPC-MLV (Table 2). Furthermore, using MLV composed of a mixture of miscible PCs enables fitting MLV to suit a wide range of temperatures. For example, the ratio of DMPC/DPPC can be adjusted\(^{42}\) to have a phase transition at all physiological temperatures occurring in different conditions of OA.

Discusson

This study is aimed at better understanding of the role played by phospholipid bilayers in biolubrication. For this, various PC-based liposomes were assessed for the effect of their physicochemical characteristics on cartilage lubrication.

Phosphatidylcholines (PCs) are surface-active glycerophospholipids naturally present in cartilage and synovial fluid.\(^ {3,4,15}\) The cationic choline and anionic diester phosphate moieties, which constitute the PC zwitterionic headgroup, remain fully ionized over a broad pH range with an overall zero net charge.\(^ {1}\) When the hydrophobic region of PCs consists of two (in most cases, acyl) chains, each more than 12 C long, the PCs have a cylinder-like shape and a packing parameter,\(^ {30}\) of 0.74–1.0, and will form liposomes upon hydration.\(^ {1,2,30,45}\) All PCs used in this study have a packing parameter of ≈0.8.\(^ {30,45}\)

PC bilayers can be either in a solid-ordered (SO) phase (occasionally referred to as gel or solid phase), as exemplified in this study by DBPC, HSPC, and DPPC, or in a liquid-disordered (LD) phase (also referred to as liquid crystalline or fluid phase).\(^ {42}\)

as exemplified in this study by DMPC and DOPC (Table 1). The transition from SO to LD phase is an endothermic, first-order process.\(^ {46}\) \(T_m\) is dependent mainly on the PC hydrocarbon chain composition.\(^ {1,2,31}\) In the LD phase (but not in the SO phase), the zwitterionic PC headgroups are highly hydrated.\(^ {1,2,47}\)

We focused on PCs as cartilage lubricants for three main reasons: (i) PCs are major components of synovial fluid,\(^ {3,4,15}\) (ii) upon hydration, PCs spontaneously form bilayers which assemble into vesicles, and (iii) PC headgroups in the LD phase are highly hydrated,\(^ {1,2,47}\) and therefore are expected to facilitate “hydrophilic” lubrication, which was proposed by Klein and coworkers to be the main mechanism of joint lubrication.\(^ {28,48}\)

Screening various PC-based MLV as cartilage lubricants identified DMPC-MLV as the most effective single-component lubricant under various loads and temperatures (Table 2 and Figure 2). DMPC has saturated linear 14-carbon length acyl chains and a \(T_m\) of 23.2 \(^\circ\text{C}\) (Table 1). On the basis of the above, we hypothesized that DMPC-MLV superiority is explained by the fact that at 24 and 37 \(^\circ\text{C}\) DMPC is in the LD phase and therefore its headgroup is highly hydrated. Furthermore, the PC with the shortest acyl chains capable of forming stable liposomes results in formation of the mechanically “softest” bilayers in comparison to other single-component PC bilayers used in this study.\(^ {2}\)

To evaluate this hypothesis, we modified DMPC-MLV by introducing 33 mol% cholesterol into its bilayers, to form DMPC/cholesterol-MLV. This induces a physical transition from the fluid and “soft” LD phase to the “dry” and more rigid liquid-ordered (LO) phase,\(^ {49}\) as shown by the lower bilayer compressibility \((K)\) of DMPC/cholesterol liposomes in comparison to that of DMPC liposomes (see Results section). By testing static and dynamic friction coefficients, DMPC/cholesterol-MLV was found to be a less effective cartilage lubricant than DMPC-MLV (Figure 3). Similarly, DBPC, HSPC, and DPPC liposomes, which are all in the solid-ordered (SO) phase, were found to have lower compressibility (see Results section) and were less effective lubricants than liposomes having lipid bilayers in the LD phase (Table 2, Figure 3).

The effect of the bilayer physical phase, hydration level, and bilayer \(T_m\) on lubrication efficiency was further tested by comparing friction coefficients of DMPC-MLV \((T_m = 23.2 \, ^\circ\text{C})\), DPPC-MLV \((T_m = 41.4 \, ^\circ\text{C})\), and DMPC/DPPC-MLV \((0.6/1.0, \text{mol/mol}, \, T_m = 34 \, ^\circ\text{C})\). At 37 \(^\circ\text{C}\), DMPC/DPPC-MLV was a better lubricant than DMPC-MLV (both in the LD phase); however, at 24 \(^\circ\text{C}\), DMPC/DPPC-MLV (in the SO phase) was a less effective lubricant than DMPC-MLV (in the LD phase). DMPC-MLV (in the SO phase at 24 and 37 \(^\circ\text{C}\)) was the least effective lubricant.

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associated with PC headgroups in the LD phase may act like nano ball-bearings, facilitating low friction by a hydrophilic lubrication mechanism.28,29,48.

To further study the effect of hydration on lubrication, 5 mol% of the lipopolymer mPEG-DSPE was introduced into DMPC-MLV, forming DMPC/mPEG-DSPE-MLV. The PEG moieties, extending 4–10 nm from the vesicle surface (depending on the polymer chain state, being either in a mushroom or brush configuration34), are highly hydrated (3–4 water molecules per ethylene oxide group)40 and highly flexible. However, the addition of mPEG-DSPE to DMPC liposomes did not improve lubrication over DMPC-MLV (Figure 3), which seems to be contradictory to the role of hydration in lubrication. This discrepancy is explained by the fact that the PEG moiety is nonionic, and therefore, its mode of hydration differs from the hydration mode of the zwitterionic ionized PC headgroups.29,40 It must be noted that PEG moieties may be beneficial under physiological conditions, as the extended PEG can protect liposomes from interacting with macromolecules of the interstitial fluid, similarly to the cartilage-SAPL protective role of HA.13,19

Investigating the effect of vesicle size and lamellarity on their performance as lubricants showed that large (> 0.8 μm in diameter) multilamellar vesicles (MLV) are superior to small (< 100 nm) unilamellar vesicles (SUV) (Figure 3). We propose that DMPC-MLV are superior lubricants to DMPC-SUV due to MLV retention near the cartilage surface, as a result of their size, while DMPC-SUV penetrate deeper into the cartilage, as demonstrated by PC distribution along cartilage depth (Figure 4). This could explain the fact that friction coefficients of cartilage lubricated with DMPC-SUV are only slightly lower than those of cartilage lubricated with the medium, HB. The difference in PC distribution along cartilage depth is explained by the presence of ~100-nm gaps between collagen fibers in cartilage,50 preventing deep penetration of large particles into cartilage.50,54

The reasons for the mode of distribution of DMPC-SUV deep into the cartilage are not yet fully understood. However, two aspects may be involved: one, related to the liposome small size and flexibility52 (when in the liquid disordered phase); and the other related to the cartilage structure, especially its hydrogel-like nature, and the inhomogeneous distribution of pores within cartilage.50,53,54

In addition to the main increase in PC content near the cartilage surface, in cartilage lubricated with DMPC-MLV, a trail of high PC content (in comparison to cartilage lubricated with DMPC-SUV or HB) extends between 200 and 550 μm into cartilage (Figure 4). This is explained by the fact that MLV are heterogeneous in size, being contaminated by smaller uni- and oligolamellar vesicles, which can penetrate deeper than the MLV into cartilage. SUV, on the other hand, are rather homogeneous in size (< 100 nm). This difference in homogeneity of size distribution stems from the difference in methods of their preparation.32 In vivo, microanatomy may also play an important role in the preference of MLV larger than 0.5 μm, as they are expected to be selectively retained within the synovial cavity and therefore should have long-term effects as joint lubricants.55 The prolonged retention of MLV was first described (for sheep joints) by Edwards et al.56 and was also confirmed by us in our preliminary experiments in rabbits (Dolev et al., unpublished results).

The multilamellar nature of DMPC-MLV may contribute additional advantages which are similar to those proposed by Schwarz et al.,57 explaining why multilayered nanoparticles made of tungsten disulfide added to liquid lubricants show exceptional tribological effects. Accordingly, for such large particles, van der Waals forces induce particle deformation and delamination of the external layers of the multilamellar particles, resulting in superior lubrication. Applying similar principles to cartilage lubrication suggests that when deposited on cartilage, MLV in the LD phase may deform under load and then delaminate. The highly hydrated delaminated lipid bilayers are expected to coat opposing cartilage surfaces, thus facilitating “hydrophobic” lubrication, while most of the MLV remain in the medium acting as a reservoir for PC bilayers which can replace PL bilayers coating the cartilage surfaces that undergo wear.

A previously proposed mechanism of joint lubrication suggested that a phospholipid monolayer, adsorbed to each of the opposing cartilage surfaces, forms a lipid bilayer that connects between the two cartilage surfaces. The friction plane will be in the middle of the hydrophobic region of the lipid bilayer where the two opposing hydrocarbon chains “touch” each other, a mechanism which resembles “classical hydrophobic (oil) lubrication”.43 However, in the synovium this mechanism should be thermodynamically unfavorable due the aqueous nature of the SF. Therefore, we propose that a hydrophilic lubrication29 should be more suitable. Accordingly, a single phospholipid bilayer (PC, in our system) rather than a monolayer is adsorbed (or built) on each of the opposing cartilage surfaces (which is a more thermodynamically favorable situation). The plane of friction will be the area of contact between the highly hydrated PC phosphocholine headgroups, which act as a nanobearing, reducing friction29 and cartilage wear.

Finally, comparing friction coefficients obtained by different media (saline, ISF, and low ionic strength HB) demonstrated that HB is superior to saline and to ISF (Table 2 and Figure 3). Furthermore, the total PL concentration of cartilage specimens lubricated with HB was nearly twice that of cartilage lubricated with ISF (which was similar to OA cartilage) and substantially higher than that of cartilage lubricated with saline (Figure 5), suggesting that HB may better retain naturally occurring cartilage PL, thereby improving lubrication. The superiority of HB over saline (Table 2, Figure 3) may be explained by its lower ionic strength, which induces a less compact and “softer” PL packing of the lipid bilayer, enabling rapid bilayer recovery and/or replacement after frictional events,1,58 thus further supporting the importance of bilayer softness as a major contributor to effective lubrication.

On the basis of this study, we propose that intra-articular injections of DMPC-MLV or DMPC/DPPC-MLV may be used to improve cartilage lubrication in joint dysfunctions. This was supported by the superior lubricating ability of ISF enriched with DMPC-MLV over ISF (without added lubricants), or HB alone, or HA in HB (Figure 3). The feasibility of efficacious treatment of joint lubrication deficiencies by MLV composed of DMPC or DMPC/DPPC is further supported by two recent findings: (i), the long (> 400 h) retention of MLV in sheep knee SF, post intra-articular injection,56 confirmed by our similar preliminary results in rabbit joints (Dolev et al. in preparation) and (ii), the unique

anti-inflammatory effects of PCs, blocking pro-inflammatory signaling.59 The fact that PC-MLV preparation is simple, reproducible on a large scale basis and economical, as well as the fact that PC-MLV are chemically and physically stable (see last part of Results section), makes them attractive for further preclinical and clinical development as cartilage lubricants. In addition, this study demonstrates the relevance of basic physicochemical phospholipids’ properties to their biological functions.

Acknowledgment. This work was supported in part by the Barenholz Fund and by the Philip and Joseph Gurwin Fund for Scientific Advance at the Technion (To I.E.). A.S. acknowledges support from the Barenholz Fund, A.M. thanks the McCutchen Foundation for partial support. The authors thank Prof. Nahir for supplying synovial fluids, Dr. Ellen Wachtel and Prof. David Segal for advice, and Mr. Sigmund Geller for editing the manuscript. A. Schroeder and S. Sivan contributed equally to this work. The role of Prof. D. Nitzan in initiating the interaction that led to this research is greatly appreciated.

Abbreviations

OA, osteoarthritis; PL, phospholipids; MLV, multilamellar vesicles; SUV, small unilamellar vesicles; HSPC, hydrogenated soy phosphatidylcholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DBPC, 1,2-dibehenoyl-sn-glycero-3-phosphocholine; HA, hyaluronic acid; HB, histidine buffer; SF, synovial fluid; ISF, inflamed synovial fluid; PL, phospholipids; PC, phosphatidylcholine; SAPL, surface active phospholipids; LO, liquid ordered; SO, solid ordered; LD, liquid disordered.