COMMUNICATION

Liposomes tethered to a biopolymer film through the hydrophobic effect create a highly effective lubricating surface†

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Liposomal coatings are formed on films of a biopolymer, hydrophobically modified chitosan (hm-chitosan), containing dodecyl groups as hydrophobes along the polymer backbone. The alkyl groups insert themselves into the liposome bilayer through hydrophobic interactions and thus tether liposomes, leading to a densely packed liposome layer on the film surface. Such liposomal surfaces exhibit effective lubrication properties due to their high degree of hydration, and reduce the coefficient of friction to the biologically-relevant range. The compliancy and robustness of these tethered liposomes allow retention on the film surface upon repeated applications of shear. Such liposome coated films have potential applications in biolubrication.

Phospholipid vesicles known as liposomes have been studied extensively as biomembrane mimics,[1,2] and are of much interest in applications related to drug and biomolecule delivery.[3,4] In the recent literature, liposomes, either in solution[5] or adsorbed onto surfaces,[6,7] have been reported to be efficient boundary lubricants at physiologically relevant conditions, exhibiting very low coefficients of friction (COF). Such low COF values are attributed to the lubrication ability of the highly hydrated phospholipid head groups exposed at the vesicle outer surfaces.[5,6] For example, the phosphocholine head group moiety can attach up to 15 rapidly relaxing water molecules[8–11]

leading to the concept of a hydration based lubrication.[12,13] In this context, the water of hydration can sustain large compression without being squeezed out from the gap between surfaces in shear, while at the same time allowing the hydration shells to relax rapidly, ensuring a fluid like response on shear.[12]

In this paper, we report a novel concept of fabricating films of a specific biopolymer (hydrophobically modified chitosan, hm-chitosan) that interact with liposomes through the hydrophobic effect of hydrophobe insertion into liposomal bilayers,[14,15] to tether liposomes on the film surface. We show that such tethered liposomal surfaces are robust and exhibit excellent lubrication properties reducing the COF values to between 10^{-2} and 10^{-3}, at pressures up to 158 MPa, significantly higher than the contact pressures reported in the human hip joint (up to 18 MPa).[16,17] The biocompatibility and antimicrobial properties of chitosan[18–20] additionally make these systems potentially applicable as materials for synovial joint lubrication.

Fig. 1 illustrates the concepts of this paper. Fig. 1a shows the structure of hm-chitosan used in this work where about 2.5% of the amine groups on the chitosan backbone are substituted with C-12 alkyl groups. The synthesis procedure follows that reported in the literature,[15,21] and involves the addition of aldehyde to an acidic chitosan solution in a water–ethanol mixture, followed by the addition of sodium cyanoborohydride. The detailed procedure can be found in the ESI† section. ^{1}H NMR (ESI Fig. S1†) confirms the presence of alkyl groups on the chitosan backbone. Films of hm-chitosan were prepared by evaporating aqueous solutions of hm-chitosan in 1% acetic acid (to sustain solubility) containing glutaraldehyde as a cross-linking agent. Briefly, 1 mL of 0.5% (wt/v) hm-chitosan in 1% (v/v) acetic acid solution was mixed with 0.0015 mL 10% (wt/v) glutaraldehyde, and the solution was mechanically stirred for 30 s in order to be homogeneous; then the mixture was dropped on a 22 mm × 22 mm cover glass for drying at room temperature for at least 24 hours. Our hypothesis as shown in Fig. 1b, was that upon formation of the film, there would be a sufficient number of exposed hydrophobes on the surface of the film that are able to attach to liposomes through insertion into the lipid

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† Electronic supplementary information (ESI) available: Details on NMR spectroscopy study of hm-chitosan structure, contact angles of water droplet on hm-chitosan film, cryo-TEM images of DPPC liposomes, complete data from the tribometer showing the friction force, coefficient of friction as a function of increasing load and the plot of the coefficient of friction when shearing a glass probe *versus* hm-chitosan/liposome film in 2% DPPC liposome solution. See DOI: 10.1039/c4sm01692k
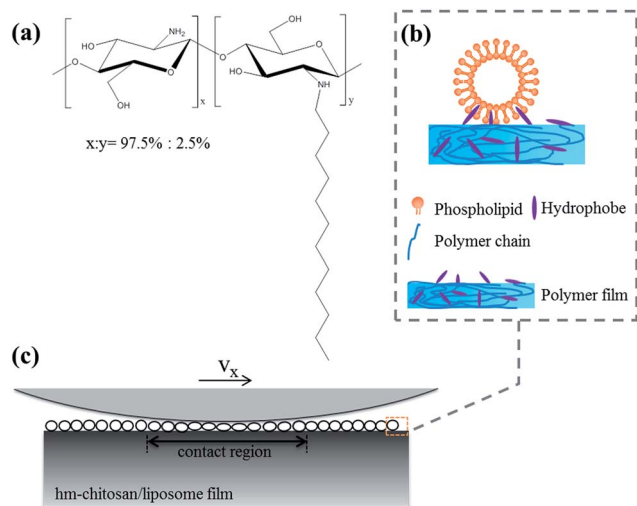
Fig. 1 (a) hm-Chitosan molecular structure; (b) schematic of hm-chitosan film tethering liposomes by inserting its alkyl groups into the liposomal bilayer (c) schematic illustrations of the contact region between a glass probe with one flat and one outward curved face and an hm-chitosan/liposome film. v_x is the fixed probe velocity.
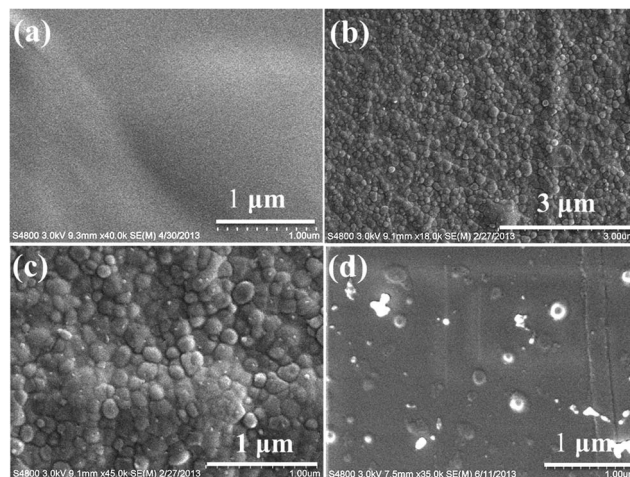

Fig. 2 Cryogenic scanning electron microscopy (cryo-SEM) images of (a) the hm-chitosan film, (b) and (c) hm-chitosan/liposome film after being washed by PBS buffer, showing a layer of close-packed liposomes on the film surface, and (d) chitosan/liposomes film after being washed by PBS buffer showing only a few liposomes on the film surface.

bilayer, in accordance with earlier studies from Raghavan's laboratory.[15,22,23] While native chitosan crosslinked films are hydrophilic (contact angle 47.2°) the use of hm-chitosan increases the contact angle to 56.8° possibly indicating an exposure of hydrophobic groups on the surface (data in ESI Fig. S2†). Fig. 1c shows the next length scale of lubrication characterization, where immobilized liposomes are placed in the contact zone between the hm-chitosan/liposome film and a glass probe surface.

The details of the study follow. Subsequent to the preparation of hm-chitosan films, they were placed in an aqueous suspension of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes. To prepare DPPC liposome, 0.1 g DPPC (Avanti Polar Lipids, Inc) was dissolved in a chloroform and methanol mixture (2 : 1 v/v) and dried under low pressure in a rotary evaporator (Buchi R210) to obtain a thin lipid film. The dried lipid film was hydrated with 5 mL of a PBS buffer solution at 50 °C for 30 min, and the suspension was then extruded through polycarbonate membranes, first with a 400 nm membrane and then with a 100 nm pore size membrane at a temperature between 55 °C and 65 °C, using an Avanti-Extruder (Avanti Polar Lipids, Inc). The cryogenic transmission electron microscopy (cryo-TEM) image of the liposome solution is shown in ESI Fig. S3.† The hm-chitosan films were incubated in DPPC liposome solution for 30 min, and then washed with phosphate buffered saline (PBS, pH = 7.4) 3 times to remove the free and loosely attached liposomes on the film. The results of this exposure of the hm-chitosan film to liposomes are shown in Fig. 2. Fig. 2a shows a bare hm-chitosan film with a smooth surface prior to incubation with the liposome solution. Fig. 2b and c illustrate the film after 30 min incubation with liposomes where it is clear that the liposomes are intact and densely packed. The dense packing of the tethered layer leads to distortions from sphericity as also observed by Klein and

coworkers for liposomes physically adsorbed on mica.[7] We note that the liposomes on hm-chitosan are tethered through hydrophobe insertion as extensive washing of the surface film has no effect on the integrity of liposome packing on the surface. Additionally, as Fig. 2d indicates, films of native chitosan without the alkyl hydrophobes are unable to capture liposomes and only a few liposomes are adsorbed to the chitosan surface after washing. Thus we attribute the tethering of liposomes to the hm-chitosan film surface as due to the hydrophobic effect wherein the alkyl groups on the polymer backbone insert into the lipid bilayer.

In order to demonstrate the functional lubrication properties of these tethered films, a commercial universal materials tester (UMT, CETR, Campbell, CA) was used to measure the friction force between the hm-chitosan/liposome film and a glass probe (radius of curvature = 3 cm, Anchor Optics, Barrington, NJ) with a curved optically polished surface (Fig. 3a). The hm-chitosan/liposome film was fixed on the bottom holder. A glass probe was attached to a force sensor (DFM-0.5, CETR, Campbell, CA) through a cantilever (spring constant $k_{DFM} = 4113$ N m^{-1}) and the movement in x and z direction was controlled by a motion actuator. The film and the probe were immersed in a PBS buffer solution for testing. A typical measurement consisted of applying an initial preload, shearing the surfaces at a fixed velocity v_x and distance, increasing the normal load, and repeating the shear cycle. The data was collected and analyzed digitally. Details of the experiment and the complete data from this equipment can be found in the ESI.†

Fig. 3b shows plots of the friction forces F_x between two shearing surfaces as a function of the normal loads L, the slope of which is the COF. Details of a complete experiment showing the F_x, L and COF values as a function of time for each cycle are in the ESI section (Fig. S4†). It is seen from Fig. 3b that the
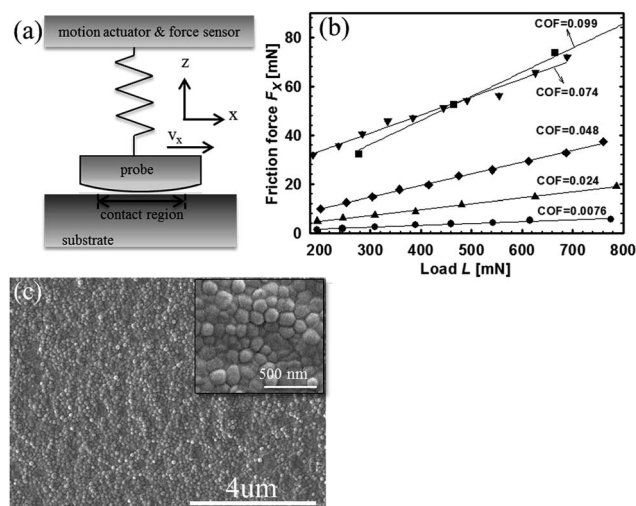
Fig. 3 (a) Schematic of the universal materials tester used to measure the friction force showing the contact region between the probe and the lubricant material, (b) plot of the friction force F_x versus the applied load L while shearing a glass probe versus a bare hm-chitosan film (▼ triangle down), chitosan/liposome film (▲ triangle up) and hm-chitosan/liposome film (● circle) in PBS buffer solution; and plot of the friction force F_x versus the applied load L while shearing a glass probe versus a flat glass surface using a DPPC liposome solution (2% wt/v) (◆ diamond) or DI water (■ square) as a lubricant. The measurements were performed with an increasing load from 196 mN (20 g) to 784 mN (80 g), equivalent to pressures from 30 to 120 MPa, sliding velocity of 1 mm s^{-1}, and dwell time of 5 s. (c) Cryo-SEM image of an hm-chitosan/liposome film after shearing a glass probe against the film at 980 mN (100 g) load for 50 cycles in PBS buffer solution show no significant wear on either the film or the liposomes.

hm-chitosan film coated with a close packed liposome layer provides a very low COF of 0.0076 in the range of COF values observed for synovial fluids.[24,25] The data indicates that over the range of loads applied, Amonton's first law of friction where the frictional force is directly proportional to the applied load, is applicable. To confirm the reproducibility of the COF value of this system, three liposome/hm-chitosan samples were fabricated and evaluated. In all cases the COF was highly reproducible with a COF value of 0.0076 ± 0.0003. As a control experiment, the friction experiment was performed with a chitosan film after incubation in a liposome solution. This system provided a COF of 0.024. The higher COF is attributed to the relatively few liposomes adsorbed on the chitosan film surface. As another control experiment, a liposomal solution was used as the lubricant between two shearing glass surfaces, resulting in a COF of 0.048; in the absence of liposomes in solution the COF was 0.099. Finally, when the hm-chitosan film and the glass probe were sheared in the absence of liposomes, a high COF (0.074) was generated. These experimental results confirmed that close-packed tethered liposomes play a major role as lubricants to reduce the COF. As an additional experiment we added liposomes in solution to the system of immobilized liposomes on hm-chitosan film, but did not observe any appreciable reduction in the COF (data in ESI Fig. S5†).

Thus, the tethering of liposomes to hm-chitosan through the hydrophobic effect leads to a dense packing of liposomes on an hm-chitosan film and exhibits lubrication properties that are in the biologically-relevant range. However, the effectiveness of a lubricant is not only measured by its ability to provide a low COF but also by its ability to reduce surface wear.[26,27] In other words, it is important that the densely packed liposomal layer is retained on the surface upon repeated applications of a shear force. Fig. 3c illustrates the cryo-SEM images of the hm-chitosan/liposome film surface after shearing a glass probe against the hm-chitosan/liposome film for 50 shearing cycles at a constant high load of 980 mN (equivalent to pressure of 158 MPa, higher than the physiological pressures in joints which are up to 18 MPa).[16,17] We observed that the liposomes remained intact and closely packed on the hm-chitosan film surface indicating a robustness of the tethering process. Throughout the cycles studied in this experiment, the COF remains at 0.0076 (data in ESI Fig. S6†).

Conclusions

In summary, we have shown that an hm-chitosan film can tether a close-packed liposome layer on the film surface via hydrophobic interactions between hm-chitosan and liposomes. The tethering of liposomes to hm-chitosan is easily accomplished and represents a facile method to capture and immobilize liposomes. The hm-chitosan/liposome film significantly reduces the COF and minimizes surface wear. The consistent lubrication properties of the hm-chitosan/liposome film are attributed to resilient hydrophobic interactions between the hm-chitosan film and liposomes. These interactions maintain a robust close-packed liposomal layer on the film surface allowing hydration lubrication over extended wear cycles. In addition to the effective lubrication properties shown here, the ease of liposome immobilization through such tethering based on the hydrophobic effect, leads to several applications in drug delivery and in fundamental investigations of biomembranes using captured liposomes and other vesicular entities.

Acknowledgements

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Supporting Information

Liposomes Tethered to a Biopolymer Film through the Hydrophobic Effect Create a Highly Effective Lubricating Surface

R. Zheng,[a] J. Arora, [a] B. Boonkaew,[a] S. R. Raghavan,[b] D. L. Kaplan,[c] J. He,[d] N. S. Pesika[a]* and V. T. John[a]*

Synthesis of hm-chitosan

4 g of chitosan was dissolved in 220 mL of 1% (v/v) acetic acid, and then 150 mL ethanol was added to allow the aldehyde used in the alkylation step to be in a solvating medium. The pH was adjusted to 5.1 by the addition of sodium hydroxide, and then a solution of dodecyl aldehyde in ethanol was added at a concentration that 2.5% of the monomer concentration. This is followed by the addition of an excess of sodium cyanoborohydride (3 mol/mol sodium cyanoborohydride/chitosan-monomer). The mixture was stirred for 24 hours at room temperature and the final product was first precipitated with ethanol and sodium hydroxide solution, and then was washed with ethanol and deionized (DI) water three times. The molecular structure of hm-chitosan was characterized through ^{1}H NMR spectroscopy to verify the attachment of the hydrophobes. The experiments were conducted in deuterium oxide using a Bruker Avance 500 MHz NMR spectrometer.

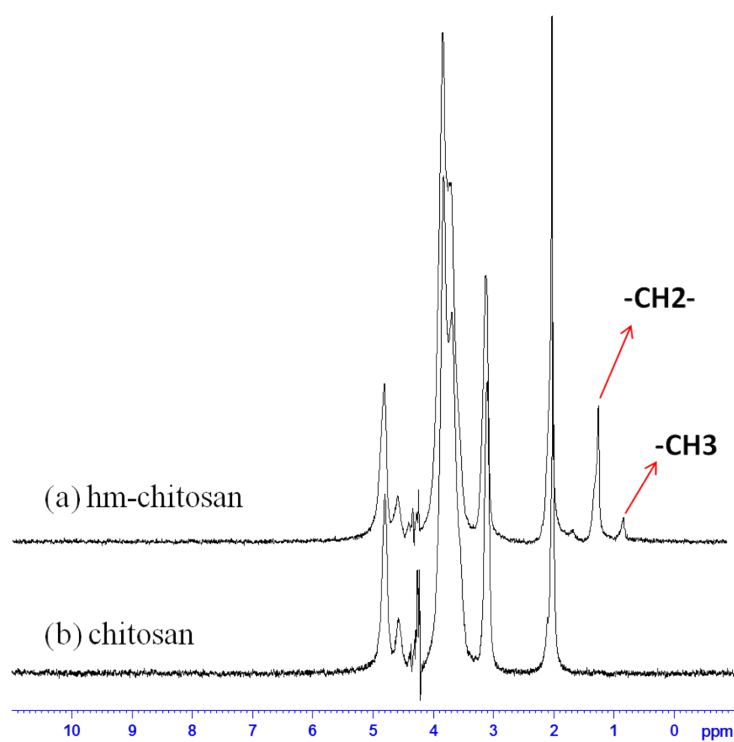**¹H NMR Spectroscopy of hm-chitosan:**

Figure S1. ¹H NMR spectroscopy of 0.50% (a) hydrophobically modified chitosan (hm-chitosan) and (b) chitosan samples indicates the successful addition of alkyl groups to the chitosan backbone.

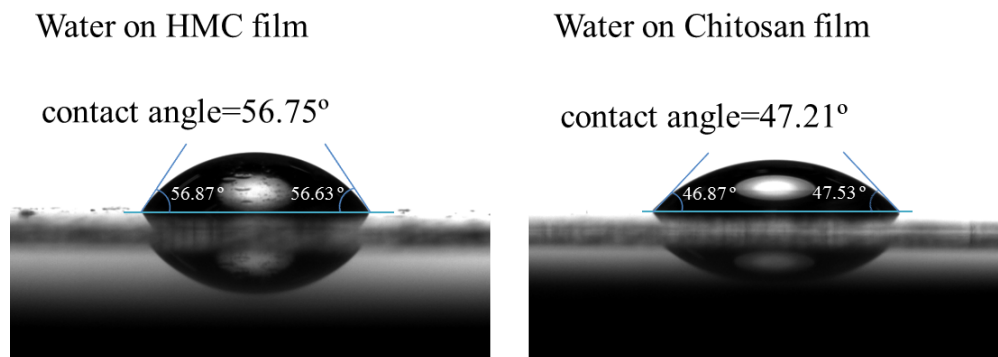**Contact Angles of Water Droplet on hm-chitosan Film:**

Water on HMC film

contact angle=56.75º

56.87º   56.63º

Water on Chitosan film

contact angle=47.21º

46.87º   47.53º

Figure S2. The contact angles of a water droplet on (a) hm-chitosan film and (b) chitosan film. The increase in contact angle with hm-chitosan is representative of the increase in hydrophobicity with exposure of alkyl groups.
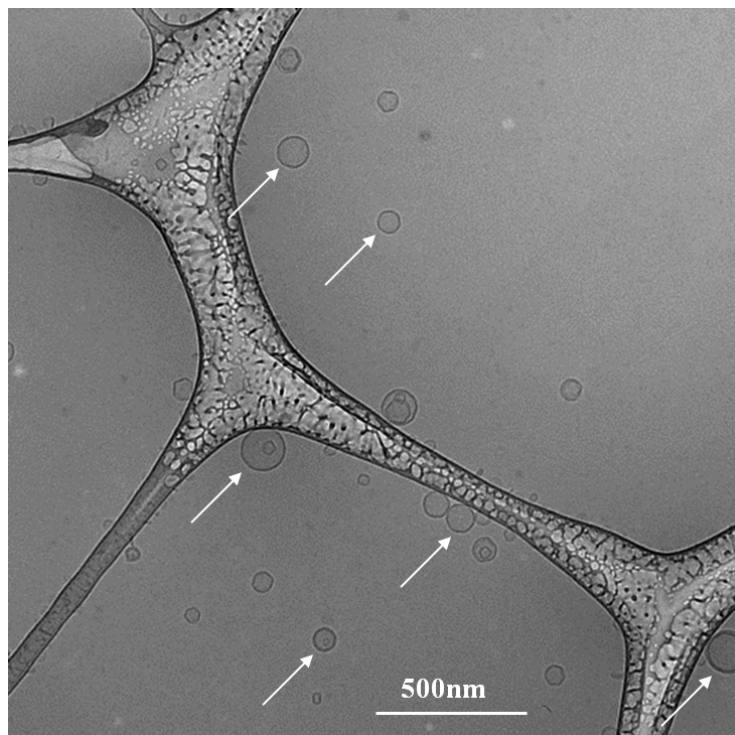
Cryo-TEM Image of DPPC Liposomes:

Figure S3. Cryogenic transmission electron microscopy (cryo-TEM) image of prepared DPPC liposomes.

Friction Measurements:

The experiments were performed with a cover glass (Fisherfinest Premium Cover Glass, Fisher Scientific), chitosan/liposome film and hm-chitosan/liposome film as the bottom shearing surfaces and a curved optically polished glass surface (radius of curvature = 3 cm, Anchor Optics, Barrington, NJ) as the probe (top surface). The glass surface was cleaned by sonication in ethanol for 5 min, followed by rinsing with DI water and a subsequent plasma-cleaning step (Harrick Plasma, Ithaca, NY). In a typical experiment, the bottom surface, either a cover glass or a prepared film, was glued onto a holder and a drop (~50 μL) of the DPPC liposome (2%, wt/v) suspension or PBS buffer solution was placed between the bottom surface and the probe. The probe, attached to a force sensor (DFM-0.5, CETR, Campbell, CA) with a cantilever (spring constant k_{DFM} = 4113 N/m), was then brought into contact with the bottom surface at a predetermined preload. A universal materials tester (CETR, Campbell, CA) was used to measure the friction force between the shearing surfaces as the load was either held constant or increased stepwise with each shear cycle. The COF was determined by taking the slope of the average friction force versus the average load for each shear cycle.

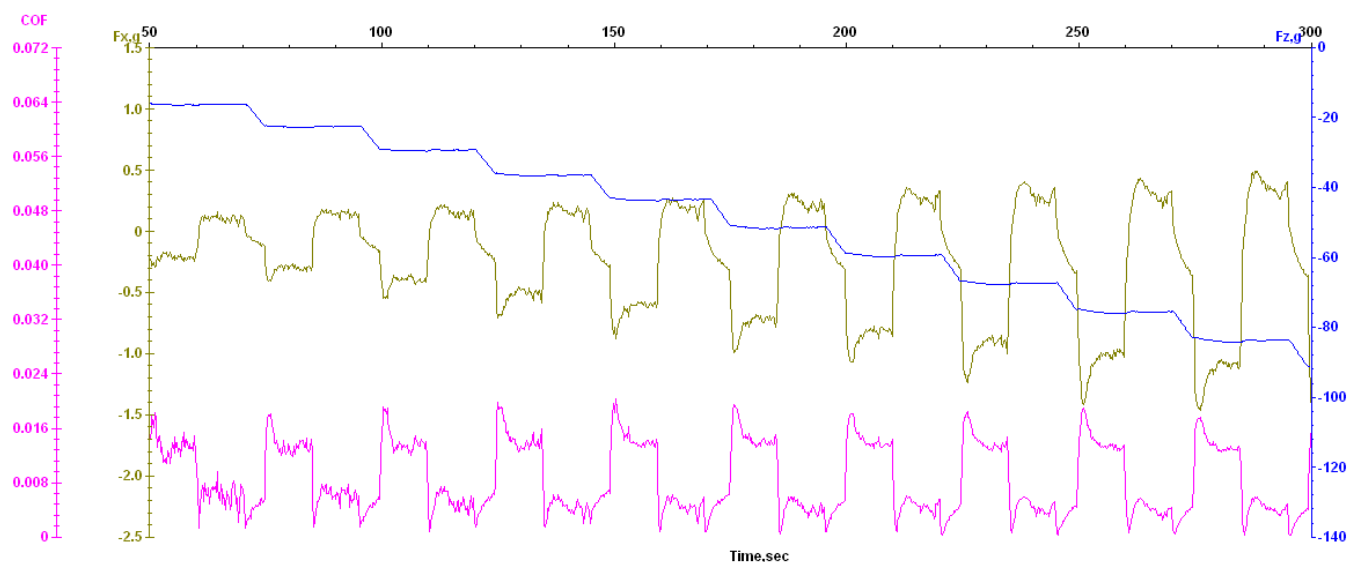**Complete Data of the Coefficient of Friction Measurement:**

Figure S4. Complete data from the universal materials tester (tribometer) showing the friction force (gold), and the COF (pink) as a function of increasing load (blue). The measurements were performed with a stepwise increasing load from 196 mN (20 g) to 784 mN (80g), a sliding velocity of 1mm/s, and a dwell time of 5s. The specific measurement shown is for the system of hm-chitosan film containing tethered liposomes in contact with a glass probe. We note that the COF remains constant at 0.0076.

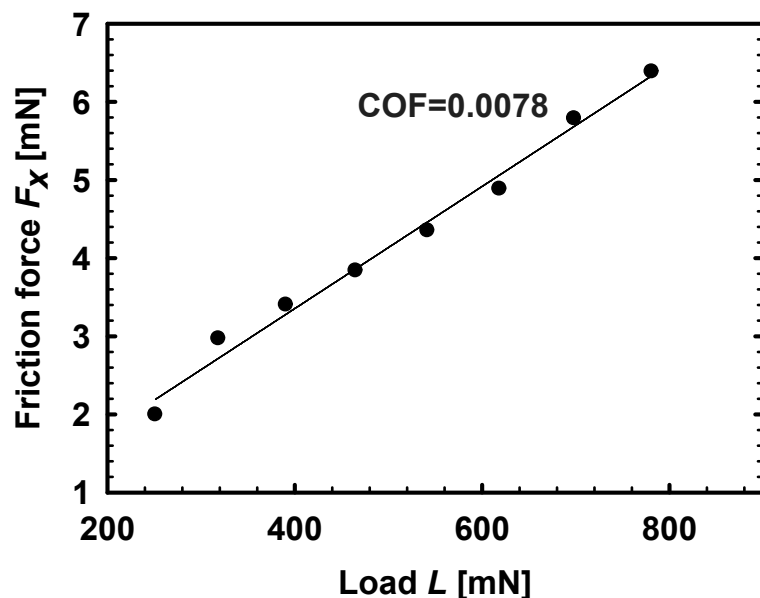**Coefficient of Friction Between hm-chitosan/liposome Film and Glass Probe in 2% DPPC Liposome Solution:**

Figure S5. The plot of the friction force F_x versus the applied load L while shearing a spherical glass probe versus hm-chitosan/liposome film in 2 % (wt/v) DPPC liposome solution. The measurements were performed with an increasing load from 196 mN (20 g) to 784 mN (80g), sliding velocity of 1mm/s, and dwell time of 5s. The data indicates that the addition of liposomes in solution does not affect the lubrication properties of the film.

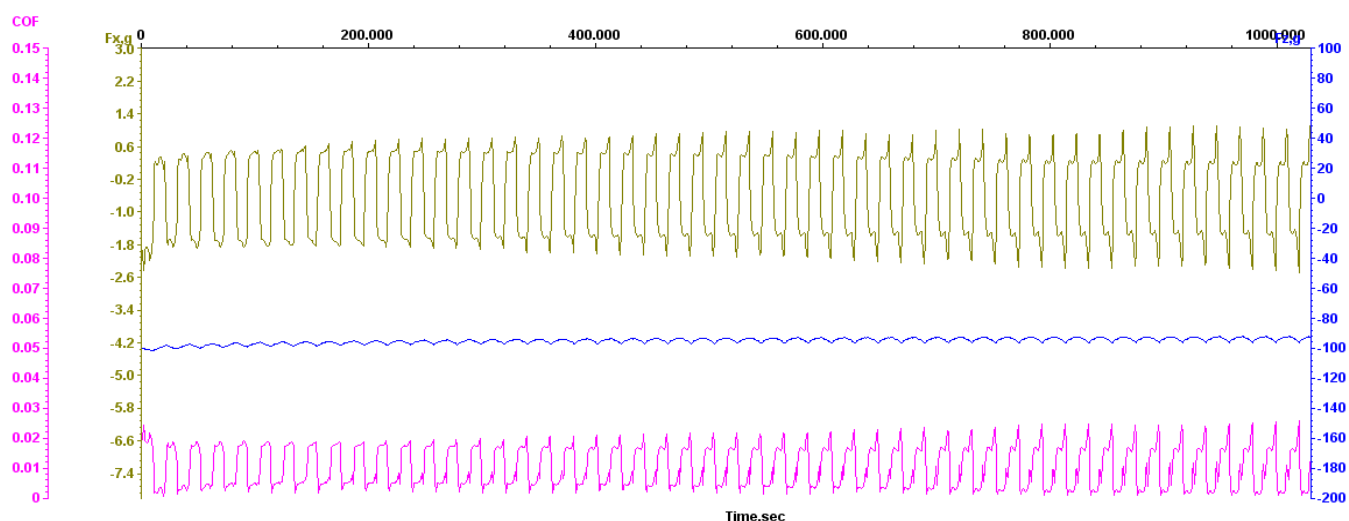**Complete Data of the Wear Test:**

Figure S6. Complete data from the universal materials tester (tribometer) showing the friction force (gold, F_x, g), and the COF (pink) as a function of increasing load (blue, F_z, g). The measurements were performed with a constant load of 980 mN (100g), a sliding velocity of 1 mm/s. The specific measurement shown is for the system of hm-chitosan film containing tethered liposomes in contact with a glass probe. The COF value remains stable during the test.