LARGE MULTILAMELLAR LIPOSOMES FORM A STABLE LAYER ON CARTILAGE SURFACE UNDER EX-VIVO PHYSIOLOGICAL COMPRESSION

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INTRODUCTION

Surface active phospholipids (SAPLs) are part of the natural boundary lubricant of articular cartilage and are degraded during progression of osteoarthritis (OA). MM-II is a suspension of large (3-4μm), multilamellar liposomes composed of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). It is administered via intra-articular (IA) administration and is currently in a phase 2b clinical study. MM-II liposomes have previously been shown ex-vivo to reduce cartilage wear and have been shown in a rat OA model to slow down cartilage degeneration. Additionally, when radiolabeled MM-II liposomes were injected into healthy rabbit knees, radio-labeling of the articular cartilage surface was detected for several weeks post injection.

OBJECTIVE

The aims of the current study were (a) to test if physiologically relevant compression load is an essential contributor to MM-II liposomes` ability to coat the cartilage surface and (b) to assess the persistence of this coating and its lubricative potential.

METHODS

Cartilage binding test under compression load

- Rhodamine-labeled MM-II liposomes (Rh-MM-II) were prepared using 16:0 Liss Rhod PE (Sigma).
- Articular cartilage discs (diameter=3mm) and synovial fluid (SF) were taken from a porcine joint.
- Three cartilage discs were glued to a rigid cylinder that was connected to a customized test-rig with a self alignment mechanism to allow optimal contact of all discs with the glass surface. The discs were held upside-down and submerged into a lubricant chamber set at +37^{0}C on a glass surface (**Figure 1**).

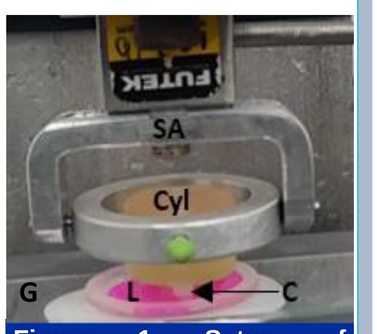


Figure 1. Setup of compression test-rig. Cyl: Cylinder, SA: Self Alignment, C: Cartilage disc, L: Labeled liposomes, G: Glass

- Compression protocol consisted of 1200 compression cycles at 1Hz, with a load ranging from 0.01MPa to 1MPa. The control group had an identical setup but without compression.
- Cartilage binding by Rh-MM-II was assessed under 3 conditions: (**A**) Discs submerged in 100% Rh-MM-II but without compression (**B**) Discs submerged in 100% Rh-MM-II under compression (**C**) Discs submerged in a mixture of 50% Rh-MM-II : 50% SF under compression.
- After each experiment, the discs were removed and thoroughly washed with PBS to remove any unbound liposomes, and frozen for histology.
- Frozen discs were sectioned into 8μm-thick coronal cryosections. DAPI staining was done for visualization of cell nuclei and images were captured using fluorescent microscopy.

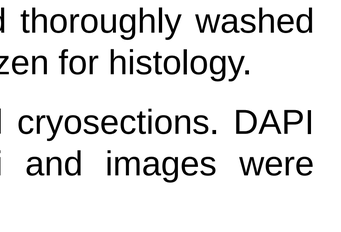
Friction tests

- Cartilage-on-glass friction tests (N=4) were performed using porcine cartilage discs (diameter=6mm) held upside-down and submerged into a lubricant chamber set at +37^{0}C on a glass surface (**Figure 2**).
- Testing was performed using a customized two-axis tribometer with a lockable self alignment mechanism. The tribometer allows the movement of the horizontal glass plate in the 3 planes to adjust the contact with the cartilage and provides high resolution measurement (0.1 mN) of friction forces.

Figure 2. Setup of cartilage-on-glass friction test in the presence of liposomes. H: Holder, SA : Self alignment mechanism, C: Cartilage disc, G: Glass counter surface, L: liposomes

- Each friction test consisted of 5 sets (**Table 1**) wherein each set in-turn consisted of 10 repetitive cycles. The design of each cycle is shown in **Figure 3**.

Figure 3. Friction test cycle.
1= Approaching glass; **2**= cartilage loading against glass for 5 sec (0.18MPa); **3**= Horizontal movement (1mm/sec over 20 mm); **4**= dwelling 0.5 sec; **5**= disconnecting; **6**= dwelling 0.5 sec; **7**=back to start point



- In order to test the ability of the liposomes layer on the cartilage surface to reduce friction in a persistent manner, friction was measured sequentially in the presence of PBS, liposomes and 3 additional sets, each time in the presence of fresh PBS (**Table 1**).

Table 1. Design of the Friction Testing Study

Sequential Friction Sets and Treatments	Lubricant
Set 1	**PBS**
Replacement with MM-II	
Set 2	**MM-II**
Cleaning of chamber & Replacement with fresh PBS	
Set 3	**PBS**
Cleaning of chamber & Replacement with fresh PBS	
Set 4	**PBS**
Cleaning of chamber & Replacement with fresh PBS	
Set 5	**PBS**

RESULTS

Cartilage binding test (**Figure 4**)

- MM-II's large multilamellar liposomes bound to articular cartilage and formed a layer on its surface which was resistant to thorough washing **(Figure 4A)**. This layer was more pronounced when the liposomes were applied to the cartilage under physiologically relevant cyclic compression **(Figure 4B)** and was evident also when the liposomes were diluted 1:1 (volume per volume) in synovial fluid to mimic a scenario whereby following intra-articular injection into a knee joint, the liposomes are mixed with pre-existed synovial fluid **(Figure 4C)**.

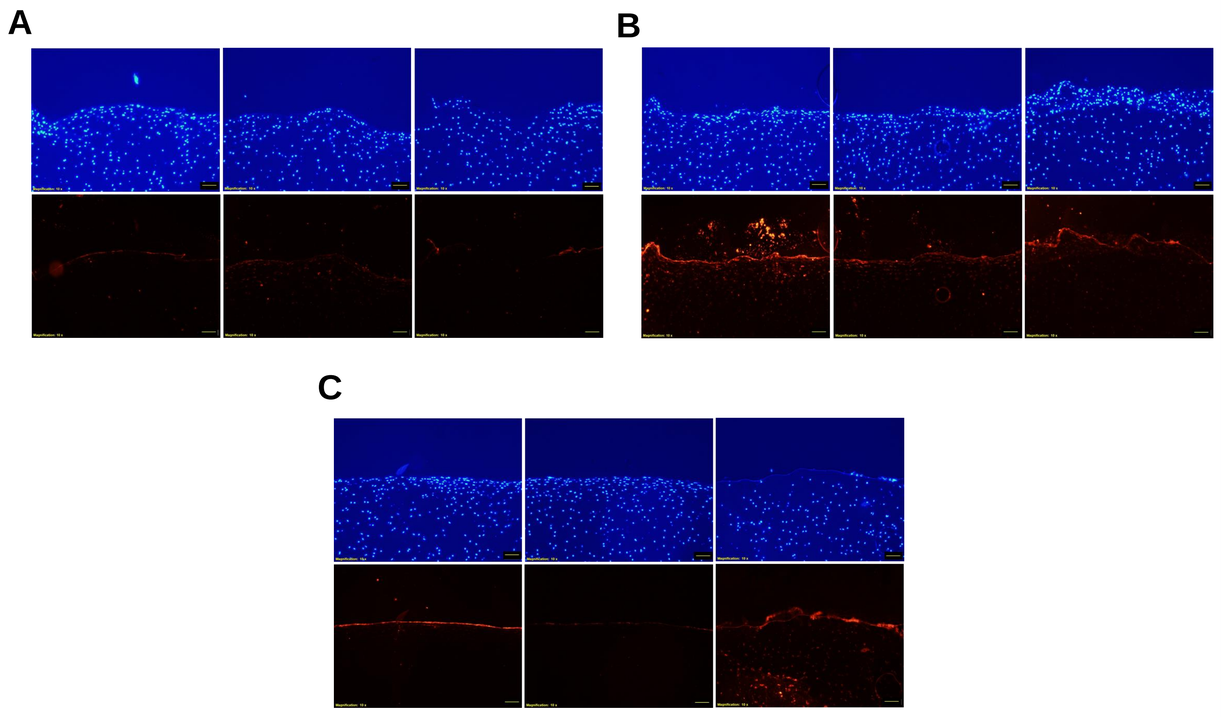


Figure 4. Binding of Rh-MM-II liposomes to articular cartilage (A) Cartilage discs were submerged in Rh-MM-II with no compression (B) Cartilage discs were submerged in Rh-MM-II under compression (C) Cartilage discs were submerged in a mixture of Rh-MM-II and SF under compression. Upper panel: DAPI staining; Bottom panel: Rhodamine signal. Scale bar: 50μm

Friction study **(Figure 5)**

- CoF_{static} and $CoF_{dynamic}$ were lowered by approximately 73% and 70%, respectively, in the presence of MM-II liposomes as compared to PBS.
- MM-II's lubricating effect was evident even after 3 successive replacements of liposomes bulk solution with PBS indicated by lowering of CoF_{static} and $CoF_{dynamic}$ by 31% and 16%, respectively.

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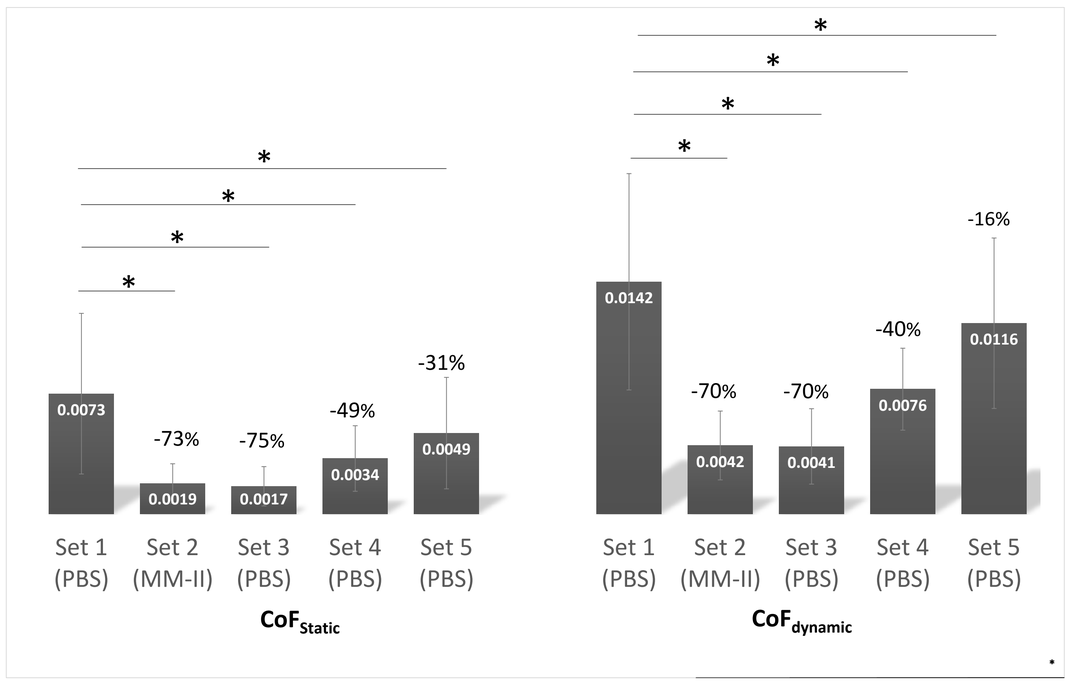

Figure 5. Sustained lubrication of MM-II.
Cartilage-on-glass friction tests (N=4) were performed on porcine cartilage discs (1 disc per test). Each disc was submerged sequentially in PBS (set 1), MM-II liposomes (set 2) and 3 additional sequential tests in presence of fresh PBS (set 3-5). The average CoF_{static} and $CoF_{dynamic}$ and the standard deviations are indicated. CoF data was analyzed using repeated measures mixed model (SAS® MIXED procedure). P-values are adjusted for multiplicity using Dunnett procedure (* p≤0.0001).

CONCLUSIONS

We show here that (a) large multilamellar liposomes form a layer on the surface of articular cartilage when exposed to physiological compression ex-vivo and (b) this layer provides lubrication even after washout of unbound liposomes. **These findings suggest that the natural biomechanical forces acting on articular cartilage in the knee joint contribute to the ability of the MM-II liposomes to provide persistent lubrication.**

CONTACT

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