Lubricin-Mimetics to Prevent Progression of Osteoarthritis

M. Tan1, KJ Samaroo1, M. Demange2, M. Sisto2, X. Deng2, SA Rodeo2, LJ Bonassar1 and D. Putnam1

1Cornell University, Ithaca, NY, 14853, USA; 2Tissue Engineering, Repair, and Regeneration Program, The Hospital for Special Surgery, New York, NY, USA.
mt525@cornell.edu

ABSTRACT SUMMARY
A study of the effectiveness of synthetic polymer brushes that mimic lubricin structure and function was investigated. In vitro binding and boundary mode lubrication tests confirm effective binding (time constants < 40 mins) and significant reduction in friction coefficients when compared with recombinant lubricin. An in vivo study using the rat ACLT model of osteoarthritis (OA) demonstrates significant reduction in friction coefficients of lubricin-mimetic treated knees when compared to PBS treated knees, suggesting potential of these lubricin-mimetics to slow OA progression.

INTRODUCTION
Osteoarthritis (OA) is a leading cause of disability in adults caused by chronic and/or acute damage to articular cartilage. OA of the knee is very common in the US: 1 in 2 people will develop symptomatic OA of the knee by age 85. This number increases to 3 in 3 for obese individuals[1]. Cartilaginous tissues of the joint have limited self-repair mechanisms which complicates treatment. Most treatments involve providing symptomatic relief but do not address the root cause – the degradation of joint tissues. Other treatments developed to address tissue damage include intra-articular injections of naturally occurring biolubricants such as hyaluronic acid (HA) to supplement and prevent further wear of the cartilage. However, the clinical effectiveness of HA injection remains controversial.[2].

Lubricin is a proteoglycan present in the synovial fluid and on the cartilage surface that acts in the boundary mode of lubrication.[3]. Boundary mode lubrication is important because the highest friction occurs under these conditions. Intra-articularly administered recombinant lubricin mutant (LUB:1)[4] and full-length lubricin[5] were shown to prevent OA progression. However, lubricin is financially prohibitive as a therapy due to its extraordinarily high cost of production.

Our working hypothesis for this research is that synthetic polymer brush lubricin-mimetics can mimic the structure-function of lubricin and prevent OA disease progression. In this study, we report the synthesis of a library of lubricin-mimetics and quantify their boundary mode lubrication against cartilage in vitro to better understand their cartilage binding characteristics and boundary lubrication. An in vivo study in a rat OA model was conducted in parallel to determine effect on OA disease progression.

EXPERIMENTAL METHODS
Synthesis of lubricin-mimetics. The synthesis of the polymer brush was conducted in two steps: 1) RAFT polymerization of acrylic acid into poly(acrylic acid) and 2) conjugation of PEG side chains onto poly(acrylic acid) (pAA) via condensation to produce poly(acrylic acid)-graft-PEG (pAA-g-PEG). Briefly, poly(acrylic acid) was synthesized through RAFT polymerization in methanol with acrylic acid as monomer, 4,4'-azobis(4-cyanopentanoic acid) as initiator and 4-cyanopentanoic acid dithiobenzoate as RAFT chain transfer agent under air-tight, O2 free conditions. Molecular weight (Mn) was altered by varying the ratios of monomer to initiator. Methoxy-PEG-amine side chains were then conjugated onto pAA using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (DMTMM) as a condensing agent. This was conducted in 0.1 M borate buffer at room temperature between pH 6 and 7. The reaction mixture was then dialyzed against DI water and lyophilized to obtain a white powder. Polymers were characterized by 1H NMR and by gel permeation chromatography.

In vitro binding and lubrication. Evaluation was conducted on denuded (stripped of natural lubricin)[3] cartilage plugs taken from patellofemoral grooves of 1-3 day old bovine calves. To visualize binding, lubricin-mimetics were tagged with fluorescein-5-thiosemicarbazide, and cartilage plugs were the incubated in solutions of fluorescent lubricin-mimetics. Binding was determined through fluorescent confocal microscopy. Frictional testing was conducted using a custom built tribometer in collaboration with Bonassar Laboratories[3] on denuded cartilage plugs incubated with non-fluorescent polymers. Testing was conducted at 0.1mm/sec shear speed and 40% compressive strain to attain boundary lubrication mode.

In vivo analysis. Sprague-Dawley rats underwent anterior cruciate ligament transection (ACLT) in each hind to simulate OA. After one week, each hind leg received an injection of either 1X PBS or lubricin-mimetic dissolved in PBS once per week for 3 weeks. After another 3 weeks the rats were sacrificed and their cartilage analyzed through frictional testing. Friction tests were conducted at shear speeds ranging from 0.1-10 mm/sec to observe any potential changes in friction regime. The study was double-blinded and randomized.

RESULTS AND DISCUSSION
In vitro results. For these studies presented, four different lubricin-mimetics were tested with varying backbone (pAA) molecular weights, side chain (PEG)
molecular weights and different grafting ratios ([PEG]/[AA]). Table 1 shows the polymer binding time constants of the fluorescently labeled lubricin-mimetics to cartilage. The nomenclature is pAA(a)-g-PEG(b) where a is Mn of pAA in kDa/mol, g is the grafting ratio of reaction ([PEG]/[AA]), and b is Mn of PEG in kDa/mol. The binding time constants were determined by comparing fluorescence with incubation time of cartilage in solutions containing each lubricin-mimetic. Binding time constants range from 21-39 min.

Table 1: Binding time constants of lubricin-mimetics

<table>
<thead>
<tr>
<th>pAA(a)-g-PEG(b)</th>
<th>Binding Time constant (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAA(60)-2-PEG(2)</td>
<td>21</td>
</tr>
<tr>
<td>pAA(60)-2-PEG(10)</td>
<td>30</td>
</tr>
<tr>
<td>pAA(105)-2-PEG(2)</td>
<td>35</td>
</tr>
<tr>
<td>pAA(105)-2-PEG(10)</td>
<td>39</td>
</tr>
</tbody>
</table>

Denuded cartilage explants were incubated in pAA-g-PEG solutions (3 mg/ml) for varying times (0-120 mins).

Calculated from general equation $z = c(1 - e^{-t/\tau})$, where $c$ are constants; $t$ is incubation time; $\tau$ is binding time constant; and $z$ is response (fluorescence or friction coefficients). $R^2 > 0.9$ for all fits.

The in vitro lubrication results are given in Fig 1. Positive controls of recombinant lubricin mutant (LUB:1) and full length lubricin were included. Three of the polymer brushes differed significantly from the unlubricated cartilage. In addition pAA(60)-2-PEG(2) and pAA(105)-2-PEG(10) reduced friction significantly compared to LUB:1, suggesting their potential as OA therapeutics. However, none of the polymers are as of yet comparable to full length lubricin.

Figure 1: In vitro lubrication results compared with an untreated cartilage as a control and LUB:1 and lubricin for comparison. Unlubricated and polymer brush results were averaged from 4 samples each and presented as the coefficient of friction ($\mu$). *(p<0.01) indicates significant difference compared to unlubricated while **(p<0.01) indicates significant difference compared to LUB:1.

In vivo results. Fig 2 shows preliminary results from an in vivo rat study using pAA(60)-2-PEG(2) lubricin-mimetic. Here rat knees injected with PBS were compared to treated rat knees injected with lubricin-mimetics.. The lubricin-mimetic vastly reduces friction across the speed spectrum when compared to PBS treated knees (p<0.002). This further supports the potential of lubricin-mimetics as a cost effective therapeutic for slowing OA disease progression.

CONCLUSION

This study investigated the design and application of lubricin-mimetics to reduce boundary mode friction on cartilage and as a therapeutic treatment for OA. The results suggest that the lubricin-mimetics not only effectively bind to denuded cartilage explants but that their binding time constants (20-39 mins) are much less than synovial clearance time (~5 hours)[6]. In addition, in vitro lubrication tests show that at least two of the lubricin-mimetics, pAA(60)-2-PEG(10) and pAA(60)-2-PEG(2), reduce friction significantly compared to LUB:1. LUB:1 was shown to prevent OA progression in rat model of OA[4]. The in vivo study conducted on the rat ACLT model of OA further shows that the lubricin-mimetic pAA(60)-2-PEG(2) significantly reduces friction in the boundary mode compared to PBS treated rat knees. These results demonstrate the potential of these lubricin-mimetics as a therapeutic for treatment or inhibition of OA by reducing frictional forces on cartilage.

REFERENCES