Comparison and Characterization of In Vitro and In Vivo Treatments of Lubricin-Mimetics on Articular Cartilage

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Introduction: Lubricin, a glycoprotein found in synovial fluid and on the surface of articular cartilage, is the primary boundary lubricant of cartilage[1] and has demonstrated the ability to protect cartilage when administered to joints after injury[2-5]. In diseased joints, such as those afflicted by osteoarthritis (OA), lubricin levels[5] and lubrication levels[2] drop significantly. The therapeutic potential of lubricin is apparent, but its widespread use for arthritis therapy may be limited by difficulties in production and associated high costs.

To overcome these challenges, we have previously reported on the synthesis of brush-like copolymers with molecular structures similar to lubricin (Figure 1) and their ability to lubricate and protect articular cartilage[6-8]. However, it is unclear whether the performance of these synthetic lubricants in vitro translate to in vivo chondroprotection. The goals of this study were to compare the effects of lubricin-mimetic treatments of cartilage in vitro to that of cartilage in vivo following joint injury.

Methods: A library of seven brush-like polymers were synthesized using two polyacrylic acid (pAA) backbone sizes (62 and 105 kDa) and two hydrophilic polyethylene glycol (PEG) side chain sizes (2 kDa and 5 kDa).6 Brush density was altered by using two PEG:AA feed ratios during synthesis (2:1 and 1:2). Polymers were synthesized with functional thiol end-groups for binding to cartilage surfaces[12]. To characterize the mimetics’ behavior in vitro, they were attached to denuded cartilage and friction tested. Cartilage plugs (n=4) were taken from the patellofemoral groove of 1-3 day old bovine calves. Native lubricin was removed from the explants using a 1.5 M NaCl solution.1 The polymers were dissolved in PBS (3 mg/ml). Cartilage explants were incubated in these solutions for two hours. To determine their frictional behavior, cartilage plugs were loaded into our custom tribometer and linearly oscillated in a PBS solution at a speed of 0.3 mm/s, under a 40% compressive normal strain.

To evaluate the mimetics’ behavior in vivo, a rat-knee injury model was used. The anterior cruciate ligament (ACL) of each hind leg was transected in 96 Sprague-Dawley rats. Rats were separated into 8 groups, where each group was treated with a unique lubricin-mimetic. Starting one week post-surgery, 50 μl of lubricin-mimetic solutions at a concentration of 3 mg/ml were injected intra-articularly into one knee, with the contralateral receiving PBS vehicle. The injections were repeated a total of once per week for 3 weeks, and rats were sacrificed 3 weeks after the final injection. All animal studies were conducted in compliance with the Institutional Animal Care and Use Committee. Legs from half of the rats were examined histologically, while the other half were tribometrically evaluated. Histologic samples were decalcified, embedded, sectioned, and stained with safranin-O. For tribometric evaluation, 3 mm cartilage samples were taken from the tibial plateau, one each from the medial and lateral
compartments. Samples were loaded into a custom tribometer and oscillated each explant in a PBS solution at a speed of 0.3 mm/s under a compressive normal stress of 250-300 kPa.

**Results:** A library of seven lubricin-mimetics was created by varying pAA backbone size, PEG side chain size, and the brush density (PEG:pAA). Each of these lubricin-mimetics was tested for their ability to lubricate and protect cartilage when administered in vitro and also in vivo after joint injury. In vitro friction values for the polymer-treated bovine cartilage ranged from 0.14 to 0.25 compared to 0.27 for the control group (Figure 2A). Polymer-treated cartilage from the pAA(62)-g(2:1)-PEG(2) and the pAA(105)-g(2:1)-PEG(10) groups had significantly lower friction coefficients than the PBS controls (p<0.001 and p<0.05 respectively). No other groups were significantly different. OARSI scores for the femoral surfaces of the injured rat knees treated with polymers pAA(62)-g(2:1)-PEG(2) and pAA(105)-g(1:2)-PEG(10) were significantly lower than their PBS-treated contralaterals (p<0.0001 and 0.05 respectively) (Figure 2B). No other groups were significantly different. Friction coefficients for tibial cartilage samples taken from rat-knees treated with lubricin-mimetics in vivo ranged from 0.36 to 0.41 (Figure 3). Friction coefficients from the bovine cartilage treated with lubricin-mimetics in vitro were significantly correlated with friction coefficients from rat cartilage treated in vivo (r²=0.58, p<0.05). The lubricin-mimetic pAA(62)-g(2:1)-PEG(2) had the lowest friction coefficients for both the in vitro and in vivo treatments and also demonstrated the chondroprotective ability apparent by the lower OARSI score.

**Discussion:** In this study, lubricin-mimetic polymers that effectively lubricated cartilage in vitro prevented the progression of cartilage degeneration when introduced into ACL transected rat knees. While differences in friction and chondroprotective ability between the lubricin-mimetics were observed, no inference was able to be made regarding different pAA sizes, PEG sizes, or copolymer brush density.

Friction coefficients for bovine cartilage treated with lubricin-mimetics in vitro correlated with friction coefficients for cartilage treated with the lubricin-mimetics in vivo post traumatic injury. The lubricin-mimetics that performed better in vitro were able to preserve the lubricating ability of cartilage surfaces in vivo. The lubricin-mimetic that resulted in the largest frictional drop in both the in vitro and in vivo treatments also resulted in the largest decrease in femoral OARSI scores. The two other polymers to show a difference in either in friction values or OARSI score, pAA(105)-g(2:1)-PEG(10) and pAA(105)-g(1:2)-PEG(10), were also the second and fourth lowest in vitro friction values. This suggests that there may be a dependence or threshold of friction values that correlate with lower OARSI scores and chondroprotection.

This study provides further evidence that lubricin’s ability to mitigate cartilage damage is linked to its ability to lubricate cartilage surfaces. The lubricin-mimetics created in this study mimic only lubricin’s structure, yet demonstrated the ability to both lubricate and protect cartilage. This ability was manipulated by varying the size of the components of the mimetics, and better lubricants resulted in better preservation of the cartilage tissue in injured joints evident by both the preservation of the cartilage’s lubricating surface and histologic OARSI scoring.

**Significance:** In this study, we developed a library of lubricin-mimetic synthetic lubricants that demonstrated the ability to lubricate and protect cartilage to varying degrees. Better lubricants result in better chondroprotection, providing a method for screening potential OA treatments for animal trials.
Figure 1. Lubricin structure (A) provides a template for synthetic lubricants (B).
Figure 2. (A) Friction coefficients of cartilage treated by different lubricin-mimetics in vitro. (B) OARSI scores for cartilage treated by different lubrincin-mimetics in vivo.
Figure 3. Friction coefficients of cartilage treated by different lubricin-mimetics in vitro compared to friction coefficients of cartilage treated by the same lubricin-mimetics in vivo.

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