Lubricin Mimetic Polymer Exhibits Efficient and Extended Lubrication

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INTRODUCTION: Lubricin is considered a boundary lubricant in synovial fluid (SF). It has shown promise for osteoarthritis (OA) treatment in both small and large animals[1,2,3]. However, lubricin is challenging to synthesize and purify, which has complicated its widescale clinical translation. These challenges have led to new engineering efforts towards creating synthetic boundary lubricants[4,5,6,7]. Although these synthetic lubricants have been shown to lubricate cartilage, none of these meets or exceed lubricin's lubricating ability, and have not yet been translated to the clinic. Recently, an engineered lubricin mimetic polymer, LM1, was shown to highly effectively lubricate healthy cartilage[13]. However, for LM1 to be effective for treatment of OA, it needs to be able to lubricate degraded cartilage and provide long-lasting lubrication. Previously, different cartilage degradation models, both biochemical degradation via IL-1β[11] and mechanically impacted cartilage[12], have been shown to inhibit lubrication. The purpose of this study was to assess whether LM1 lubricates both IL-1β degraded cartilage and mechanically impacted cartilage, as well as determine how long its lubricative effects are seen.

METHODS: *Polymer Synthesis*: The LM1 polymer was synthesized via sequential reversible addition−fragmentation chain-transfer (RAFT) polymerization to achieve a diblock structure. It consists of a poly(acrylic acid) backbone with distinct features in each block: a quaternary amine-decorated binding block that anchors the polymer to cartilage surfaces via charge-charge interactions, and a poly(ethylene glycol)-rich lubrication block with a bottle-brush structure that resists compression. A solid form of the polymer was solubilized in phosphate-buffered saline (PBS), resulting in a concentration of 10 mg/ml that was used in testing. *Tribological testing*: Friction characterization was performed using a previously described[8], cartilage-on-glass tribometer. Briefly, femoral condyles were dissected from neonatal bovine joints. Full-thickness 6 mm diameter cartilage plugs were biopsy-punched from the condyles and trimmed to a thickness of 2 mm. These plugs were then glued onto a brass pivot and incubated in PBS or LM1 for two hours at room temperature. Immediately following the incubation, the articular surface was mated against a polished glass surface in a PBS bath, compressed to 30% strain, allowed to stress relax for one hour, and then reciprocally slid at speeds ranging from 0.1 – 10 mm/s. The friction coefficient was determined by calculating the shear-to-normal load ratio, measured by a biaxial load cell. *IL-1β Culture:* Cartilage explants were dissected from bovine femoral condyles, biopsy punched, and trimmed, as described above. The explants were then put into DMEM media with or without a 10 ng/ml treatment of IL-1β. The explants were cultured for 7 days with media changes every 2-3 days. After culture, explants were washed in PBS to remove any residual media and then incubated in PBS or LM1 for 2 hours, and subsequently friction tested as described above. Explants that were not used immediately for friction testing were frozen and saved for later friction testing. *Mechanical Impact Injury:* Cartilage plugs were placed onto an impactor device[12], which imparted peak impact stresses of ~17 MPa and peak stress rates of ~21 GPa, shown to induce subcritical cracking of the cartilage surface[12]. After impact, the explants were incubated in PBS or LM1 for 2 hours and then friction tested. *Extended Lubrication:* To test how long the effects of the LM1 incubation are seen, cartilage explants were incubated in LM1 or PBS for two hours immediately after dissection and afterwards were placed in media and cultured for set timepoints. After the culture periods, explants were friction tested. *Statistics*: Linear mixed-effects models were applied to the degraded cartilage models with speed, lubricant, and tissue condition as fixed effects and the random effects included the trial number of the tribometer and condyle number. The "emmeans" package in R was used for post-hoc comparisons.

RESULTS: Within both control and IL-1β groups, when the cartilage explants were incubated in LM1 before sliding, the friction coefficients were significantly lower than those from a PBS incubation ($p < 0.0001$). Between the PBS incubation groups, explants treated with IL-1β during culture, had higher friction coefficients compared to untreated explants ($p = 0.0012$). Contrastingly, when explants were incubated in LM1 and then slid in PBS, IL-1β treated explants tended to have lower friction coefficients compared to control cultured explants ($p = 0.06$). (Fig 1A). In the mechanical impact study, there was again a significant difference in friction coefficients between PBS and LM1 groups in both the impacted and non-impacted groups ($p < 0.0001$). There was no difference between impacted and non-impacted samples incubated and slid in PBS, nor was there a significant difference between impacted and non-impacted when incubated in LM1 (Fig 1B). In the extended lubrication study (Fig 3C), there was a significant difference in friction coefficients between the LM1 and PBS group at each timepoint (Day 0, 5, 10: $p < 0.0001$; Day 15: $p = 0.015$). Additionally, if later timepoints are compared back the baseline of day 0, there was significant difference ($p < 0.01$) in both groups at days 10 and 15, but not at day 5. However, the PBS group has a decreasing friction coefficient over time, whereas the LM1 group increases; despite these differing trends, LM1 friction coefficients remained lower than those of PBS (Fig1C).

DISCUSSION: The incubation in LM1 prior to sliding provides the large decrease in friction seen across all groups, despite not being present in the lubricant bath, as traditional tribology would call for. Notably, in the IL-1β treatment study, the PBS group had increased friction coefficients between untreated and IL-1β treated cartilage, meanwhile the LM1 group trended towards a decrease in friction coefficients after IL-1β treatment (Fig 1A). We speculate that the cytokine challenge may compromise surface integrity, potentially exposing proteoglycans and allowing for stronger charge-charge interactions with LM1. Furthermore, LM1 lubricated mechanically impacted cartilage just as well as healthy cartilage (Fig 1B). These two observations show that LM1 has an ability to efficiently lubricate cartilage surfaces independent of the different surface properties. Additionally, there was enhanced lubrication for over two weeks after a single 2-hour incubation (Fig 1C). An experiment like this across many days, to our knowledge, has not been conducted previously, and points to the possibility of an extended residence time in the joint after injection, while still providing mechanical effects. Overall, these findings suggest that LM1 has potential to lubricate osteoarthritic cartilage and maintain mechanical effects many days after initial exposure.

SIGNIFICANCE: This study measured remarkably low friction coefficients of cartilage slid in PBS after incubation in a lubricin mimetic polymer across different cartilage surface conditions and showed decreased friction coefficients for over 2 weeks after a single incubation. These findings show the promising lubricating ability of this synthetic bottle-brush polymer that may be used to help treat osteoarthritis.

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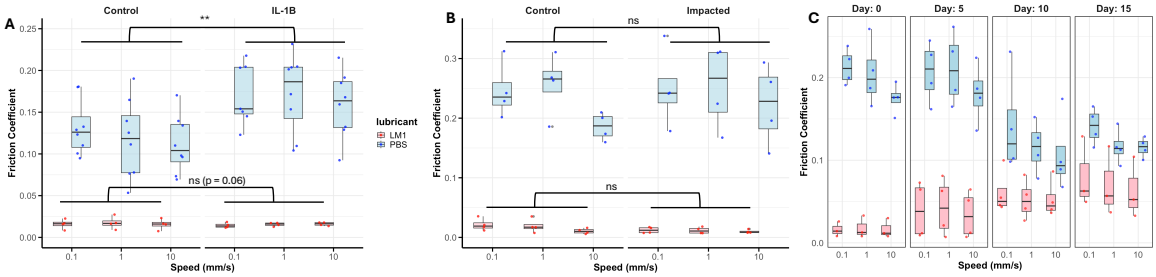

Figure 1: Friction coefficient for cartilage slid at different speeds in PBS after a 2-hour incubation in PBS or LM1. **A:** Control cultured vs IL-1B treated cartilage plugs. **B:** non-impacted and mechanically-impacted cartilage plugs. **C:** Cartilage plugs received an initial 2-hour incubation in PBS or LM1, then were cultured over many days, and immediately following the culture period, were friction tested.