Published in final edited form as:

Biomed Mater Eng. 2011 ; 21(0): 281–289. doi:10.3233/BME-2012-0676.

Human synoviocyte lubricin and bovine synovial fluid lubricin equally improve gliding resistance in a canine model *in vitro*

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Abstract

The lubricating ability of human synoviocyte lubricin and bovine lubricin purified from synovial fluid was investigated and compared using a canine *in vitro* tendon model. Our null hypothesis was that these two forms of lubricin would have equal lubricating ability. Forty two canine hindlimbs were used. The peroneus longus (PL) tendons were harvested, along with the proximal phalanx and flexor digitorum profundus of the second or fifth digit with its proximal fibro-osseous pulley. Forty PL tendons were randomly assigned to one of four treatment groups. After gliding resistance testing, two intact PL tendons and two tendons in each group were randomly selected for surface observation with scanning electron microscopy (SEM). The variance of the PL saline group mean gliding resistance was significantly different from other groups. There was a significant treatment-cycle interaction effect on the mean gliding resistance. On SEM, the surface of the saline treated PL tendons appeared rough, whereas the other tendon surfaces appeared smooth. Human synoviocyte lubricin functioned as well as bovine synovial fluid lubricin to reduce friction of canine PL tendons *in vitro*. This data suggest that treatment using the two forms of lubricin are mechanically similar.

Keywords

Human bovine lubricin; gliding resistance; flexor tendon

1. Introduction

Complex hand injuries in zone II, the region in the fingers where the two flexor tendons pass through a series of fibro-osseous pulleys, often lead to postoperative complications including adhesion formation and reduced finger mobility. In order to facilitate motion of a repaired or grafted tendon through the pulley, various lubricants have been studied for their ability to reduce gliding resistance and to prevent adhesions. The issue is particularly important when an extrasynovial tendon is used as a graft to replace an injured intrasynovial flexor tendon which cannot be repaired, since the extrasynovial tendons have a suboptimal gliding surface, with much higher gliding resistance and a much greater risk of adhesion formation [1].

Lubricin, also known as *superficial zone protein* or *proteoglycan 4* (PGR4), is an endogenous mucinous glycoprotein that functions in lubricating articular cartilage surfaces [2,3]. Lubricin may mitigate adhesion-dependent synovial growth [4], as well as protein deposition on cartilage surfaces from the surrounding synovial fluid [5–7]. Lubricin may also affect flexor tendon lubrication [8,9].

The application of a carbodiimide derivatized hyaluronic acid and gelatin (cd-HA gelatin) combined with exogenous bovine synovial fluid lubricin (b-lubricin) [10] can reduce the gliding resistance of peroneus longus tendon and the repaired flexor tendon in a canine model [1,11]. Furthermore, *in vivo* animal studies have shown that treatment with cd-HA gelatin b-lubricin is associated with a decrease in adhesion formation after flexor tendon repair [12] and peroneus longus tendon grafting [13] suggesting that lubricin could potentially be used as a therapeutic agent to improve the outcome after flexor tendon injuries. For clinical use, a source of human lubricin would be advantageous and recently such sources have become available [6,14]. Therefore, the purpose of this study was to examine the lubricating efficacy of carbodiimide derivatized gelatin and hyaluronic acid, plus dimeric [14] human synoviocyte derived lubricin (h-lubricin) relative to carbodiimide derivatized gelatin and hyaluronic acid plus bovine synovial fluid lubricin (b-lubricin) in a canine model *in vitro*. Our null hypothesis was that there would be no difference in lubricating ability between the two forms of lubricin, as measured by gliding resistance after 1000 motion cycles *in vitro*.

2. Materials and methods

2.1. Tendons and treatment

Forty-two peroneus longus (PL) tendons were obtained from the hind limbs of animals sacrificed in the course of other, Institutional Animal Care and Use Committee approved studies. In each limb, the PL tendons were excised from the musculotendinous junction to the bony insertion and the surrounding paratenon was carefully removed. In addition, the proximal phalanx of the second and fifth digit with its pulley was removed and cleared of any membranous tissue. Eight flexor digitorum profundus (FDP) tendons were also harvested from the musculotendinous junction to the bony insertion. The FDP tendons were used as a normal intrasynovial tendon control.

Forty PL tendons were randomly assigned to one of four treatment groups ($n = 10$): (1) 0.9% NaCl 0.1 M Mes saline, as a control (Sigma, St. Louis, MO, USA); (2) cd-HA, a group treated with just the carbodiimide derivatized gelatin (Sigma, St. Louis, MO, USA) with hyaluronic acid (MW 95% 1.5×10^6 , Acros, Geel, Belgium); (3) b-lubricin, bovine synovial fluid lubricin (courtesy of Gregory Jay, MD, PhD, Providence, RI, USA) at 260 $\mu\text{g/ml}$ bound to a carbodiimide derivatized gelatin of 10% gelatin with 1% hyaluronic acid and (4) h-lubricin, human synoviocyte lubricin (SBH Sciences, Natick, MA, USA) at 260 $\mu\text{g/ml}$ also bound to a carbodiimide derivatized gelatin with hyaluronic acid.

To prepare the carbodiimide derivatized solutions, EDC and NHS with hyaluronic acid (HA) was placed in a syringe. Another syringe was filled with gelatin and both syringes were connected with a common needle and both contents were ejected simultaneously into a Petri dish. Each of the treated tendons was immersed in a dish with the cd-HA-gelatin solution for 30 s. The tendons were then allowed to cure for 10 min while wrapped in a smooth rubber sheet with a saline moistened towel. After the tendon was mounted in the testing device, excess cd-HA-gelatin was removed by several initial passes under the pulley. After 5 additional cycles of flexion–extension motion, the lubricin treated tendons were immersed in a 260 $\mu\text{g/ml}$ solution of either h-lubricin or b-lubricin for 30 min.

2.2. Measurement of gliding resistance

After preparation as described above, the tendons were mounted into a custom-made frictional testing machine with the volar side of the phalanx facing upward. The testing apparatus consisted of a mechanical actuator, a linear potentiometer, two custom made tensile load transducers with ring-shaped load cells made of aluminum with a full Wheatstone bridge configuration and 350 Ω foil gauges, a pulley, a 4.9 N weight (F_1) attached to distal transducer to maintain tension on the tendon as well as a proximal load transducer (F_2) connected to a custom made mechanical actuator with a small linear slide driven by a stepper motor. The tendons were passed through the proximal pulley of the proximal phalanx and a 2-mm Kirschner longitudinal wire was drilled through proximal interphalangeal joint at full extension.

The tendons were positioned so that the proximal end of the tendon was placed at $\alpha = 30^\circ$ while the distal end of the tendon was placed at $\beta = 20^\circ$ in accordance to previous studies that utilized the same equipment [1] (Fig. 1). Once mounted, the tendons were subjected to 1000 cycles of flexion-extension motion at a rate of 2 mm/s over a 7.5 mm excursion distance, during which 54 data points of the frictional force were recorded per cycle. Throughout the mechanical testing, the tendons were immersed in a saline bath, maintained at room temperature.

In order to calculate the mean gliding resistance over each cycle the following equation was employed:

$$F_{\text{Mean Gliding Resistance}} = \frac{(F_{2\text{Forward}} - F_{2\text{Reverse}})}{2}.$$

2.3. Scanning Electron Microscopy (SEM)

After measurement of gliding resistance, two intact PL tendon and two tendons from each treatment group were randomly selected for surface observation with scanning electron microscopy. These tendon segments were fixed with Trump's solution, dehydrated, mounted on specimen stubs and coated with gold/palladium. The morphology of tendon surface was observed using a Hitachi S-4700 field emission scanning electron microscope (Hitachi, Pleasanton, CA, USA).

2.4. Statistics

The gliding resistance data at cycles 1 and 1000 from each of the five experimental groups were evaluated using the Brown–Forsythe test for homogeneity of variance and one-factor ANOVA with repeated measures, followed by a Tukey–Kramer post-hoc test. *P*-values less than 0.05 were considered to be significant. Statistical analysis was performed with JMP (SAS, Cary, NC, USA) software.

SEM specimens were assessed qualitatively.

3. Results

The gliding resistance (GR) of tendons was measured over 1000 cycles of flexion-extension motion. After a small decrease in the first few cycles, GR of untreated PL tendons increased monotonically as additional loading cycles were applied. In contrast, the GR of FDP tendons and PL tendons treated with cd-HA-gelatin with or without lubricin changed little over 1000 cycles of repetitive motion (Fig. 2). Initial mean GR ($\pm$ SD) for the FDP saline ($n = 8$), PL saline ($n = 10$), PL b-lubricin ($n = 10$), PL h-lubricin ($n = 10$) and PL cd-HA ($n = 10$) were

0.082 ± 0.011 , 0.115 ± 0.057 , 0.065 ± 0.022 , 0.056 ± 0.014 and 0.053 ± 0.007 N, respectively. After 1000 cycles of tendon motion, the GR for the FDP saline ($n = 8$), PL saline ($n = 10$), PL b-lubricin ($n = 10$), PL h-lubricin ($n = 10$) and PL cd-HA ($n = 10$) were 0.052 ± 0.010 , 0.621 ± 0.162 , 0.087 ± 0.040 , 0.070 ± 0.014 and 0.081 ± 0.036 N, respectively (Fig. 3).

Qualitative assessment of group variance was aided with dot plots of GR data (Fig. 4), which indicated PL saline GR at both time points was more widely distributed. Homogeneity of variance testing at both time points revealed group variances were not equal ($P = 0.0075$ and $P < 0.001$ for cycles 1 and 1000, respectively). Exclusion of PL saline GR data resulted in homogeneity of variance for the remaining groups at both cycle 1 and cycle 1000, with P -values of 0.2874 and 0.0765, respectively. Therefore, based on the outcome of qualitative and statistical assessment, PL saline GR was excluded from the ANOVA analysis to prevent violating the homoscedasticity requirements of the method.

Treatment resulted in a significant difference in mean GR between the data points of 1 and 1000 cycles, ($P < 0.0001$), indicating an interaction effect between these independent variables. The number of loading cycles applied created an overall significant difference in mean GR ($P = 0.0498$). Treatment resulted significant differences in the mean GR at cycle 1 ($P = 0.009$) but not at cycle 1000 ($P = 0.0763$). Tukey post hoc testing revealed that mean GR of the FDP saline group was significantly different than that of both the PL h-lubricin ($P = 0.0034$) and PL cd-HA ($P = 0.0009$) groups at cycle 1. As expected, the GR of the FDP tendon in saline solution was the lowest of all the treatment groups at 1000 cycles.

3.1. SEM evaluation

The surface morphology of intact PL tendons and PL tendons after 1000 cycles of flexion-extension motion was studied with SEM. Consistent with the GR findings, a smooth surface was observed for the intact PL tendon and the PL tendons treated with cd-HA-gelatin with or without lubricin at low magnification (Fig. 5). However, the surface of the saline control PL tendons after 1000 cycles had a rough appearance at the same magnification, with loose fibers visible on the tendon surface (Fig. 6).

4. Discussion

Animal studies have demonstrated the effectiveness of cd-HA-gelatin combined with bovine lubricin to reduce friction and adhesions. However, while lubricin has been identified in a variety of tissues in multiple species, it is unknown whether species specific variations in lubricin affect its function or therapeutic effect. In this study no significant difference in mean GR was observed between tendons treated with cd-HA-gelatin and human or bovine lubricin. This study supports a similarity in mechanical function between human and bovine lubricin, at least *in vitro*, even though one is a dimer and the other, a multimer. However, this study does not indicate that the addition of either type lubricin results in any significant improvement or detriment in GR over treating the tendon with cd-HA-gelatin alone. PL tendons treated with human lubricin did have a significantly lower mean GR than that of FDP tendons in saline, supporting the concept that such treatments to extrasynovial tendons result in mechanically acceptable substitutes for intrasynovial grafts. Although removed from the statistical analysis for reasons previously described, the mean GR of the PL saline group is clearly significantly higher at 1000 cycles than all other treatment groups, which confirms that surface treatment of PL tendon is required to create a functionally similar substitute for intrasynovial tendons and that treatments had a significant effect on the friction properties of the tendon.

Significant differences in the variances of each treatment group at both 1 and 1000 cycles were observed in this study, with the variance of the PL saline group being different in both cases. A possible explanation for this result, which seems particularly relevant for the 1000 cycle results, is that the variance is to some extent scaled with the mean for each group. Since mean GR of the PL saline group was nearly seven-fold that of the next lowest group mean, the variance should be expected to be larger.

These findings are important, because clinical studies of lubricin would likely be conducted with recombinant lubricin which is a heterodimer [6,14] similar to lubricin derived from human synoviocytes. For patients with complex flexor tendon injuries to zone II of the hand, lubricants like lubricin may significantly increase the likelihood of functional restoration of the digit. Similarly, the lubricating qualities of lubricin may be appropriate for supplemental therapeutic use or by clinicians performing complex flexor tendon procedures, such as tenolysis or tendon grafting.

Effects of some treatments on mean GR of PL tendons were different than those previously reported. Using nearly identical techniques, Taguchi et al. observed mean GR of cd-HA-gelatin treated PL tendons with and without lubricin added to be somewhat higher than the values reported in this study at 1000 cycles [1]. However mean GR of PL tendons treated with cd-HA-gelatin was slightly lower than that reported in another study [15]. We therefore conclude our results fell within a reasonable range.

Without a more detailed molecular analysis, the SEM images can only be used qualitatively. We noted was seemed to be smoother surfaces on the treated tendons. Whether this represents less wear or a filling or grouting effect of the surface treatment remains for future study.

As with any *in vitro* study, certain limitations exist that one must take into account when analyzing the results. One limitation of the study was the inability to include the PL-saline group data in ANOVA analysis as was planned due to the variance of this group being statistically different from the other. It compromised our ability to state with statistical confidence whether or not this untreated extrasynovial tendon's properties were different than the FDP initially. Furthermore, the difference in friction properties between the untreated PL group and others after 1000 cycles was evident without statistical analysis, a finding that is consistent with previous studies [1,15].

While the tendons used were immediately frozen to -80°C once they were removed from the tissue, an unforeseen modification to the tissue strength or surface micro-architecture may have influenced the outcomes of the results. Nevertheless, it is our opinion that the results derived from the mechanical tests can provide useful insight into the lubricating qualities of both analogues of lubricin. As research on lubricin's lubricating qualities are still in their infancy, *in vitro* models such as this set the foundation for further *in vivo* studies.

A second limitation is in the choice of model. While studies utilizing canine animal models are common throughout orthopedic research, differences in morphology and anatomy must be taken into account when contextualizing the results of such studies for human use. As quadrupedal animals, canines use a digitigrade stance in which the weight of their body is supported by only their phalanges. More to the point, the clinical impact of human digital tendon injuries are in the hand, which are not weightbearing structures. These inherent differences in stance and locomotion may affect the functional morphology and anatomy of the respective species, which must be taken into account when performing a biomechanical analysis such as this. Despite the differences in load bearing forces of canines and humans digits, similarities in flexor tendon anatomy make canines an ideal animal model for the

study of tendon injury, repair, reconstruction and tissue engineering, prior to conducting clinical investigations. In addition, by using two xenogenic sources of lubricin, we did not have an opportunity to study any species specificity of lubricin's effect.

A final limitation was that this was an *in vitro* study; clinical relevance will be determined only after *in vivo* studies have been performed. While no difference in mean GR was observed between groups treated with cd-HA-gelatin; cd-HA-gelatin and bovine lubricin; and cd-HA-gelatin and human lubricin, this is but one of several parameters that need to be examined to judge the effectiveness of each treatment. In future studies, it will be important to study the *in vivo* effect of human lubricin on adhesion formation as well.

5. Conclusion

In summary, our study showed that dimeric lubricin derived from human synoviocytes was as effective as multimeric lubricin derived from bovine synovial fluid in reducing canine tendon gliding resistance *in vitro*. These results, if replicated *in vivo*, also suggest that some therapeutic results of bovine lubricin, which have been extensively documented in animal models [1,11], are likely to be comparable for human lubricin.

Acknowledgments

This study was supported by grants from NIH/NIAMS (AR44391) and Mayo Foundation.

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Fig. 1.
Mechanical testing apparatus for measuring tendon gliding resistance.

Fig. 2. Changes in mean gliding resistance of all treatment groups over 1000 cycles. Bars represent standard error at each time point. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-2012-0676>.)

Fig. 3. Mean gliding resistance of the treatment groups at 1 and 1000 cycles. (A, B) Indicate statistically significant differences. *Indicates groups excluded from statistical analysis. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-2012-0676>.)

Fig. 4.
Statistical variation in mean gliding resistances at 1 and 1000 cycles.

Fig. 5.
The smooth surface of intact PL tendon and PL tendons treated with cd-HA-gelatin with or without lubricin at low magnification SEM.

Fig. 6.

The surface of PL tendon in the saline control group after 1000 cycles of flexion–extension motion had a rough appearance, with large strands twisted and loose fibers on the tendon surface. Cd-HA-gelatin was found to coat the collagen fibers, which are randomly distributed on the PL tendon surface.