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The Effect of Surface Treatment Using Hyaluronic Acid and Lubricin on the Gliding Resistance of Human Extrasynovial Tendons *In Vitro*

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Abstract

Purpose—The purpose of this study was to investigate the effects of the tendon surface treatment using hyaluronic acid (HA) and lubricin on the gliding resistance of human extrasynovial palmaris longus (PL) tendon in vitro.

Methods—Thirty two fresh-frozen human fingers and sixteen ipsilateral PL tendons were used. Each PL tendon was divided into two pieces which were randomly assigned into four experimental groups. After the gliding resistance of the normal PL tendon segments were measured, the tendons were treated with either saline, carbodiimide derivatized gelatin and hyaluronic acid (cd-HA-gelatin), carbodiimide derivatized gelatin with lubricin added (cd-gelatin+lubricin), or cd-HA-gelatin +lubricin. After treatment, tendon gliding resistance was measured up to 1000 cycles of simulated flexion/extension motion.

Results—The gliding resistance of the PL tendons in the cd-HA-gelatin, cd-gelatin+lubricin and cd-HA-gelatin+lubricin groups was significantly lower than that of the saline treated control after 1000 cycles ($p<0.05$). The gliding resistance in these treatment groups decreased within the first 50 cycles and then increased at a much more gradual rate over the 1000 cycles, with the cd-HA-gelatin +lubricin group being most stable.

Conclusion—The results suggest that tendon surface treatment using HA and lubricin can improve the gliding of human PL tendon in vitro. If validated in vivo, tendon surface treatment has the potential to improve the gliding ability of tendon grafts clinically.

Keywords

Gliding Resistance; Hyaluronic Acid; Lubricin; Tendon Surface Treatment

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INTRODUCTION

Many surgical regimens and post operative rehabilitation protocols have been developed to treat patients with finger flexor tendon injury, but restoration of finger function is still a difficult task.[1,2] When tendon repairs fail, the tendon graft plays an important role in reconstruction to restore finger function. Most tendon grafts are obtained from extrasynovial tendon sources that are easily harvested with limited risk of donor site functional loss.[3] Unfortunately, extrasynovial tendon grafts are known to develop more adhesions to the surrounding tissue than intrasynovial tendon grafts.[4–6]

Tendon gliding ability, assessed by surface friction, can influence the outcome after tendon graft and repair.[7–9] The friction of extrasynovial tendon increased significantly more than that of intrasynovial tendon with repetitive load cycles[7,10], and increased friction in repaired canine flexor digitorum profundus (FDP) tendon[8] is associated with increased adhesion formation.

Tendon surface friction is mainly affected by surface smoothness, and lubrication between the gliding surfaces. Tendon surface treatment using lubricants such as hyaluronic acid (HA), phospholipids, or lubricin can reduce surface friction and adhesions. The effect of HA on flexor tendon has been investigated in animal and clinical studies.[11–15] Although HA may prevent adhesion formation between the tendon and surrounding tissue without affecting tendon healing[16–19], in vivo results have not been consistent, possibly because unmodified HA is rapidly metabolized.[14,20,21] Tissue engineering approaches can establish a stronger bond between hyaluronic acid and the tendon surface. Carbodiimide derivatized HA (cd-HA)[22–24] improved gliding of canine fibularis peroneus longus (FL) tendon grafts over 100 cycles in vitro.[25] Gelatin combined with the cd-HA (cd-HA-gelatin) further reduced friction in canine FL tendon grafts, and the effect persisted in vitro over as many as 500 cycles.[26]

Lubricin is a mucinous glycoprotein responsible for the boundary lubrication of articular cartilage.[27,28] It has the same lubricating ability as normal synovial fluid *in vitro*. Lubricin added to a tendon surface pre-treated with carbodiimide derivatized gelatin (cd-gelatin +lubricin) can significantly reduce the gliding resistance of the extrasynovial tendon and maintain a smooth tendon surface after 1000 cycles of simulated flexion/extension tendon motion in a canine model in vitro.[29]

While tendon surface treatments using hyaluronic acid and lubricin may improve the gliding ability of a canine tendon, it is unknown whether these substances would improve the function of a human tendon. The purpose of this study was to investigate the effects of tendon surface treatment with cd-HA-gelatin, cd-gelatin plus lubricin and cd-HA-gelatin plus lubricin on the gliding resistance of extrasynovial tendon in a human model in vitro.

MATERIAL AND METHODS

Specimen Preparation

Thirty two fresh-frozen fingers and sixteen ipsilateral palmaris longus (PL) tendons were obtained from sixteen different human cadavers. The cadavers were stored at −20° and were thawed before testing. The third and fourth fingers of each hand were randomly assigned to four different treatment groups. In each finger, the A2 pulley and the proximal phalanx were preserved with removal of all other soft and bony tissues. A 1.5 –mm Kirschner were inserted through the proximal phalanx, parallel to the long axis of the bone. PL tendons were harvested from their insertion to their musculotendinous junction. As recommended clinically when extrasynovial tendons are used for tendon grafting[30,31], most of the paratenon was removed, leaving only a thin layer, so as not to damage the underlying tendon surface. Each PL tendon

was cut transversely into two pieces, proximal and distal, thus creating 32 PL segments which were randomly assigned into four experimental groups, with 8 in each group.

Tendon Surface Modification

Lubricin was purified from bovine synovial fluid as reported in a previous study[32], and preserved at −20°C until used. The purified lubricin was diluted with a solution of 0.1 M Mes (2-(N-Morpholion) ethanesulfonic acid) (Sigma) and 0.15 M NaCl to a 260 ug/ml) The PL tendons were randomly assigned to one of four treatment groups: saline control; cd-HA-gelatin; cd-gelatin+lubricin and cd-HA-gelatin+lubricin (Table 1). In the cd-gelatin+lubricin and cd-HA-gelatin+lubricin groups, the tendons were first coated with cd-gelatin or cd-HA-gelatin without lubricin, then wrapped in a smooth rubber sheet for ten minutes. Excess lubricant on the tendon surface was removed by gliding the tendon against the pulley for five cycles of simulated flexion/extension motion. The tendon was then immersed in 260 μg/ml lubricin for 5 minutes. In the saline control group, the tendon was immersed in saline for 5 minutes and then tested.

Measurement of Tendon Surface Gliding Resistance

We used a modified version of a previously described and validated testing device to measure the gliding resistance between the PL tendon and A2 pulley of the digit.[9,33] Each digit was secured on the custom-made device with the volar side upward in a saline bath (ISOTEMP 202, Fisher Scientific, Houston, TX) at 37°C. The measurement system consisted of a mechanical actuator with a linear potentiometer, two custom-made tensile load transducers, and a mechanical pulley (Figure 1). The load transducers were connected to the distal and proximal ends of the PL tendon. A 4.9-N weight was connected to the distal transducer (F1) to maintain tension on the PL tendon. The proximal load transducer (F2) was connected to a custom-made mechanical actuator with a small linear slide driven by a precision gearhead direct-current motor. Based on the experience of previous studies [33,34] a set arc of contact, 30° and 20° between the horizontal plane and the proximal and distal transducer cables, respectively, was used to measure the gliding resistance. The tendon was pulled proximally by the actuator against the 4.9-N weight at a rate of 2 mm/s. The excursion distance was 19 mm, an average excursion of the human digital flexor tendon at the A2 pulley.[7] The force differential between the proximal and distal tendon ends represents the gliding resistance of the PL tendon against the A2 pulley of the finger, which could be obtained by (F_2flexion − F_2extension)/2.

The data was initially recorded in the normal PL tendon for one cycle. After tendon surface treatment, data was recorded after every 50 cycles up to 500 cycles and then after every 100 cycles up to 1000 cycles.

Statistical Analysis

The gliding resistance prior to the surface treatment and after 1000 cycles of tendon motion was analyzed using one-way analysis of variance (ANOVA). A Tukey-Kramer post-hoc test for individual comparisons was used if there was a significant difference. A $p<0.05$ significance level was used in all cases.

RESULTS

There was no significant difference in gliding resistance of the PL tendon before treatment among the four groups ($p = 0.39$). There was also no significant difference in gliding resistance between the proximal and distal segments of the PL tendon before treatment.

After 1000 cycles of tendon motion, the gliding resistance of the PL tendon in saline, cd-HA-gelatin, cd-gelatin+lubricin and cd-HA-gelatin+lubricin groups was 0.75 ± SD (standard

deviation) 0.14 N, 0.33± SD 0.15 N, 0.26 ± SD 0.16 N, and 0.20 ± SD 0.09 N, respectively. The gliding resistance of the PL tendons in the cd-HA-gelatin, cd-gelatin+lubricin and cd-HA-gelatin+lubricin groups was significantly lower than that of saline control after 1000 cycles ($p < 0.05$). The gliding resistance of the saline control PL tendons increased 190% over 1000 cycles of tendon motion ($P < 0.05$). There was no significant difference in gliding resistance before and after 1000 cycles for the cd-HA-gelatin, cd-gelatin+lubricin and cd-HA-gelatin +lubricin treated groups (Figure 2).

The trend of gliding resistance in each group is shown in Figure 3. The gliding resistance of the saline treated control PL tendons in increased sharply over the first 50 cycles and then increased more gradually over 1000 cycles. The gliding resistance of the PL tendons in the cd-HA-gelatin, cd-gelatin+lubricin and cd-HA-gelatin+lubricin groups decreased within the first 50 cycles and then increased at a gradual rate over the 1000 cycles. The rate of change in gliding resistance per cycle of motion (i.e., the slope of the gliding resistance vs cycle number curve displayed in Figure 3) between 100 to 1000 cycles was significantly lower for the cd-HA gelatin +lubricin treated tendons than for the saline control tendons ($p < 0.05$) (Figure 4).

DISCUSSION

The human palmaris longus tendon is a common source for tendon graft clinically because the functional loss at the wrist is slight, it is in the same field as the flexor tendon, and it is easily accessible.[31] The canine fibularis (peroneus) longus tendon is commonly used for tendon grafting experimentally as there is no true canine equivalent of the palmaris longus tendon, which is only present in primates. Although, both the human PL and canine FL tendon are extrasynovial and seem to be very similar, there is a difference in the gliding resistance between the normal human PL and canine FL tendon. In a previous study, the mean gliding resistance of the human PL tendon and canine FL tendon was reported to be 0.52±0.22N and 0.09±0.03N, respectively.[7,26] There also may be differences in the response to tendon surface treatment.

In this study, the gliding resistance of the human PL tendon in the cd-HA-gelatin, cd-gelatin +lubricin and cd-HA-gelatin+lubricin groups was significantly lower than that of saline control after 1000 cycles of tendon motion. The gliding resistance in these treatment groups decreased within the first 50 cycles and then increased at a much more gradual rate over the 1000 cycles. The results suggest that tendon surface treatment using HA and lubricin can improve the gliding of human PL tendon in vitro, which is similar to the effect of tendon surface treatment on the canine FL tendon in vitro reported previously.[26,29]

The difference in gliding resistance between the groups treated with cd-HA-gelatin with or without lubricin was not significant, although there was a trend toward lower gliding resistance of the tendon treated with cd-HA-gelatin plus lubricin. It is possible that interspecimen variation may have affected these results. The irregularities of the surface of the PL tendon were partly a function of the amount of the paratenon that was removed in the course of specimen preparation, which was designed to be comparable to what is done clinically.[30,31] The rate of increase in friction over the 1000 cycles was least with the cd-HA-gelatin+lubricin tendons, but this difference was only significant when compared to the untreated tendons. A larger sample size may have confirmed a statistically significant difference, but without in vivo data it is difficult to determine if such changes might be clinically significant.

The principal limitation of this study is that it was an in vitro investigation. However, extrasynovial tendon treated with cd-HA-gelatin improved digital work of flexion and tendon gliding resistance in a canine tendon graft model in vivo.[35] Although further studies are needed, the similarity in results in the canine and human models in vitro indicates that tendon surface

treatment with hyaluronic acid and lubricin may improve the gliding ability of a human graft tendon in vivo.

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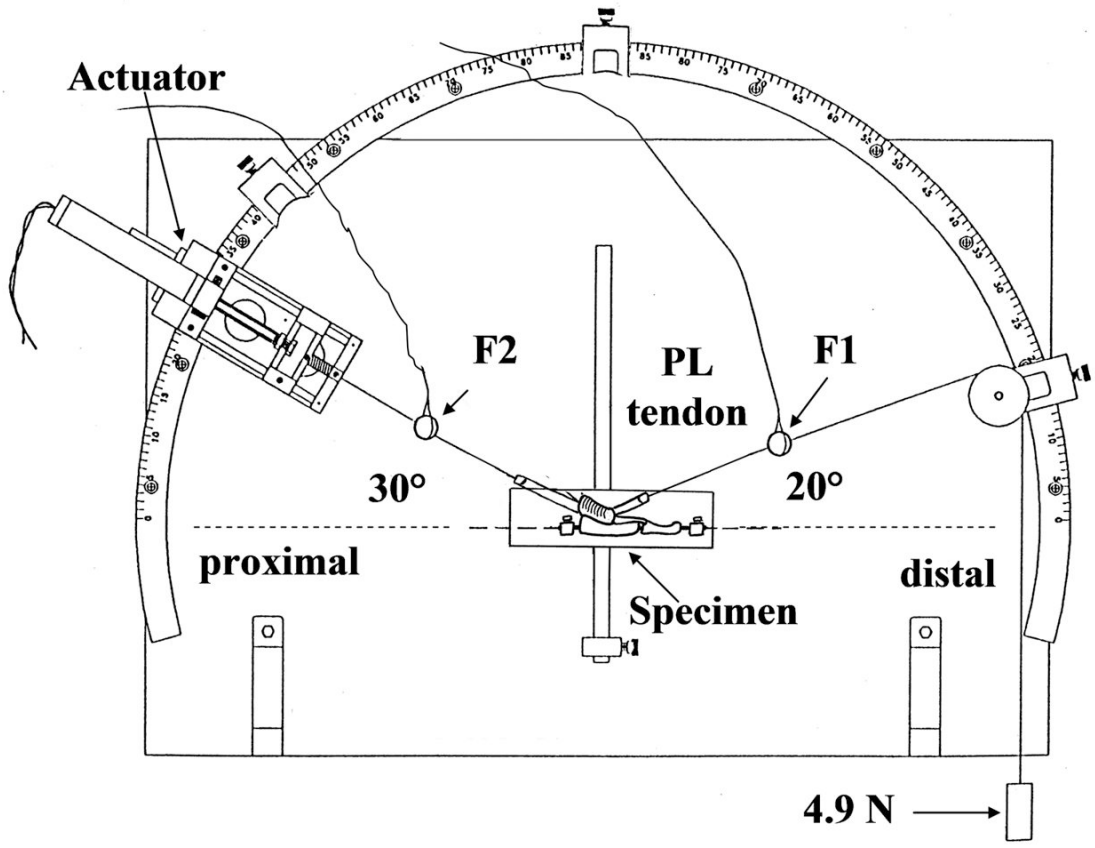

Figure 1.
Lateral view of testing apparatus for measurement of gliding resistance between PL tendon and A2 pulley. F1 is the distal force transducer and F2 is the proximal force transducer.

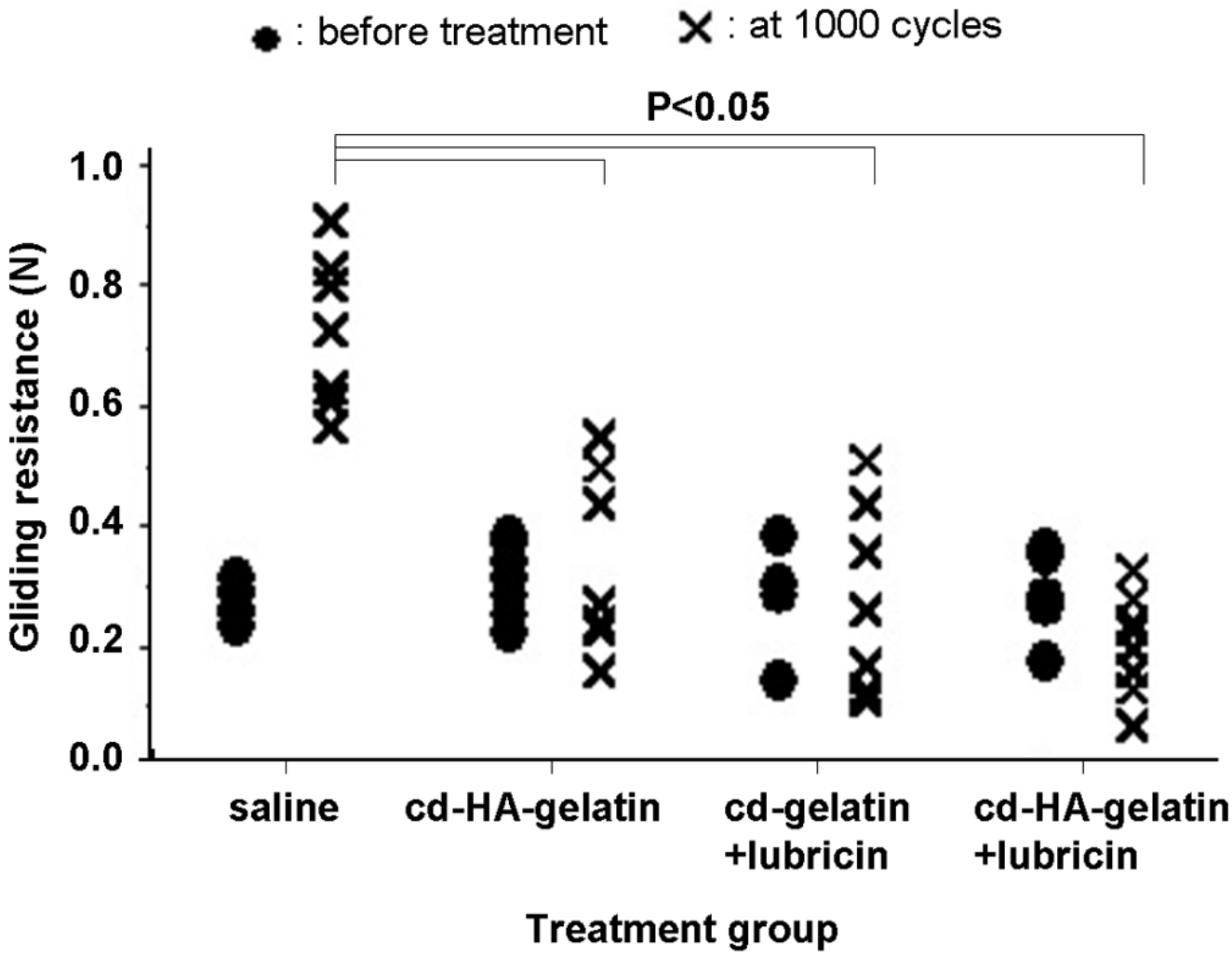

Figure 2.

Gliding resistance of PL tendon before treatment and after treatment at 1000 cycles. Tendon was treated with saline (saline), 1% HA/10% gelatin/1% EDC/1% NHS (cd-HA-gelatin), 10% gelatin/1% EDC/1% NHS + lubricin (cd-gelatin+lubricin), and 1% HA/10% gelatin/1% EDC/1% NHS + lubricin (cd-HA-gelatin+lubricin) ($p<0.05$).

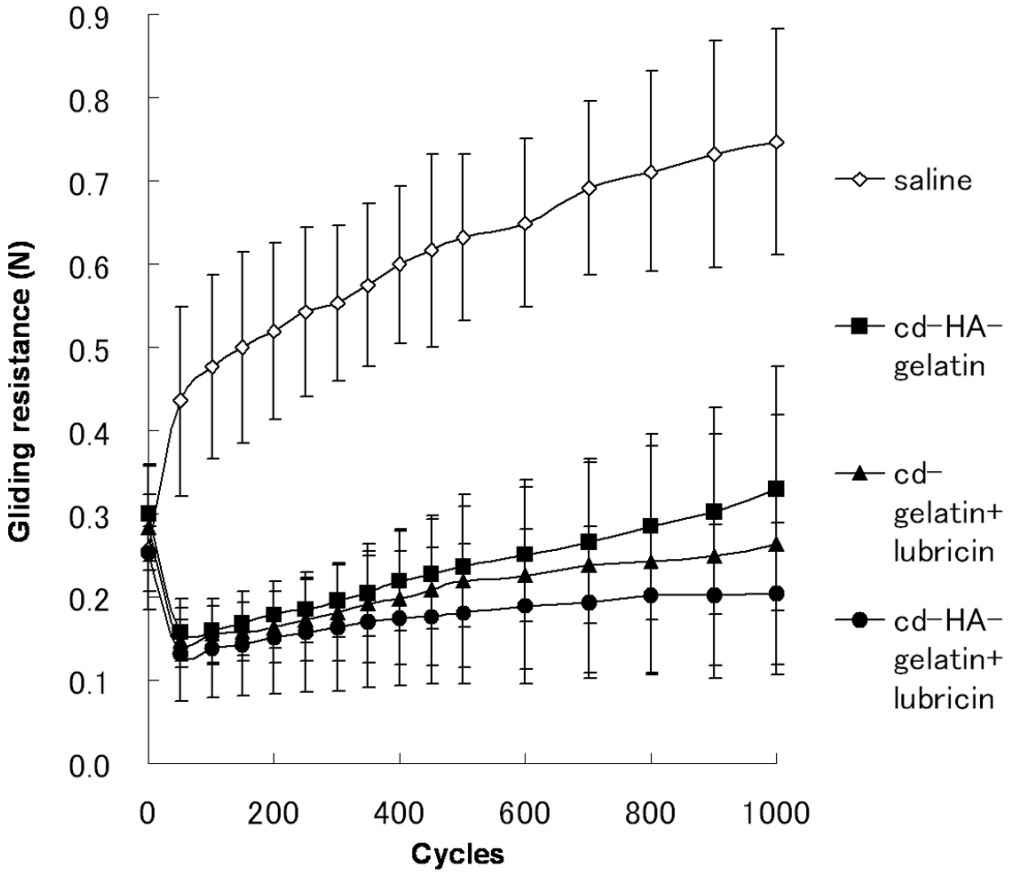

Figure 3.
Gliding resistance of PL tendon.

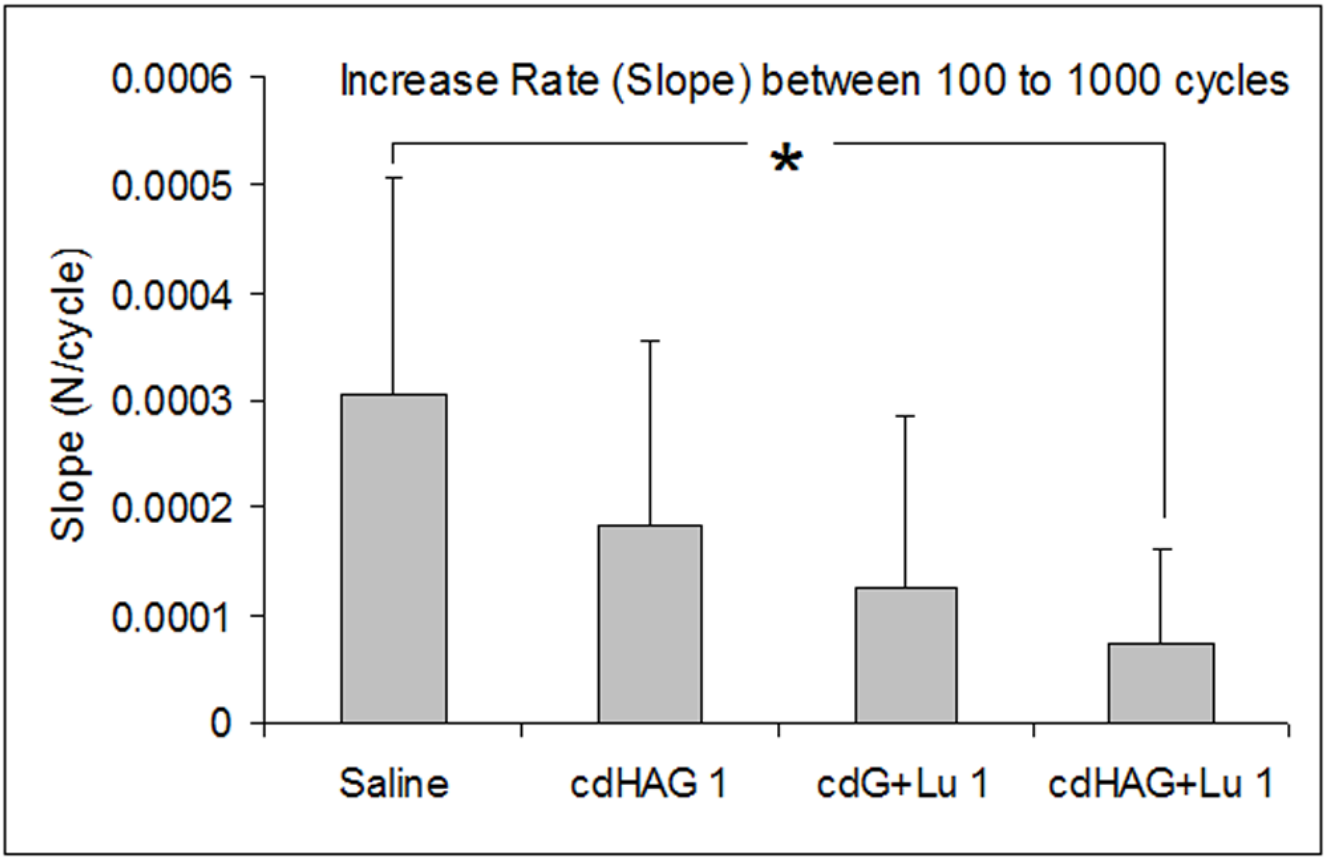

Figure 4.
Rate of change in gliding resistance between 100 to 1000 cycles in all groups. "*" indicates the difference is significant ($p < 0.-5$).

Table 1

Formulation of the Tendon Surface Treatments

Experimental Group	Formulation
saline	0.9% NaCl, 0.1 M Mes (Sigma, St Louis, MO) at pH 6.0
cd-HA-gelatin	1% sodium hyaluronate (HA) (Acros, Geel, Belgium), 95%, 1.5×10^6MW. 10% gelatin (Sigma) 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma) 1% N-hydroxysuccinimide (NHS) (Pierce, Rockford, IL) 0.9% NaCl. 0.1 M Mes pH 6.0
cd-gelatin+lubricin	10% gelatin, 1% EDC, 1% NHS, in 0.9% NaCl and 0.1 M Mes at pH 6.0 After 5 cycles of tendon motion, add 260 μg/ml bovine lubricin, 0.9% NaCl, 0.1 M Mes at pH 6.0
cd-HA-gelatin + lubricin	1% HA, 10% gelatin, 1% EDC, 1% NHS, in 0.9% NaCl and 0.1 M Mes at pH 6.0 After 5 cycles of tendon motion, add 260 μg/ml bovine lubricin, 0.9% Nacl, 0.1 M Mes at pH 6.0