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

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Purpose To investigate the effects of 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 cadaver human fingers and 16 ipsilateral PL tendons were used. Each PL tendon was divided into 2 pieces, which were randomly assigned into 4 experimental groups. After the gliding resistance of the normal PL tendon segments were measured, the tendons were treated with either saline, carbodiimide derivatized (cd) gelatin and HA (cd-HA gelatin), cd gelatin with lubricin added (cd gelatin plus lubricin), or cd-HA gelatin plus lubricin. After treatment, tendon gliding resistance was measured during up to 1000 cycles of simulated flexion and extension motion.

Results The gliding resistance of the PL tendons in the cd-HA gelatin, cd gelatin plus lubricin, and cd-HA gelatin plus lubricin groups was significantly lower than that of the saline-treated control after 1000 cycles. 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 plus lubricin group being most stable.

Conclusions 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. (J Hand Surg 2009;34A:1276–1281. © 2009 Published by Elsevier Inc. on behalf of the American Society for Surgery of the Hand.)

Key words Gliding resistance, hyaluronic acid, lubricin, tendon surface treatment.

Many surgical regimens and postoperative rehabilitation protocols have been developed to treat patients with finger flexor tendon injury, but restoration of normal finger function is still a difficult task. 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. Unfortunately, extrasynovial tendon grafts are known to develop more adhesions to the surrounding tissue than do intrasynovial tendon grafts.

Tendon gliding ability, assessed by surface friction, can influence the outcome after tendon graft and re-
pair. The friction of extrasynovial tendon increased significantly more (p < .05) than that of intrasynovial tendon with repetitive load cycles, and increased friction in repaired canine flexor digitorum profundus tendon 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. Although HA might prevent adhesion formation between the tendon and surrounding tissue without affecting tendon healing, in vivo results have not been consistent, possibly because unmodified HA is rapidly metabolized. Tissue engineering approaches can establish a stronger bond between HA and the tendon surface. Carbodiimide derivatized HA (cd-HA) improved gliding of canine fibularis (peroneus) longus (FL) tendon grafts over 100 cycles in vitro. Gelatin combined with the cd-HA (cd-HA gelatin) further reduced friction in canine FL tendon grafts, and the effect persisted in vitro for as many as 500 cycles.

Lubricin is a mucinous glycoprotein responsible for the boundary lubrication of articular cartilage. It has the same lubricating ability as normal synovial fluid in vitro. Lubricin added to a tendon surface pretreated with carbodiimide derivatized (cd) gelatin (cd-gelatin plus lubricin) can significantly reduce the gliding resistance of the extrasynovial tendon (p < .05) and maintain a smooth tendon surface after 1000 cycles of simulated flexion and extension tendon motion in a canine model in vitro.

Although tendon surface treatments using HA and lubricin might 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.

MATERIALS AND METHODS

Specimen preparation
Thirty-two fresh-frozen fingers and 16 ipsilateral palmaris longus (PL) tendons were obtained from 16 human cadavers. The cadavers were stored at −20°C and were thawed before testing. The middle and ring fingers of each hand were randomly assigned to 4 different treatment groups. In each finger, the A2 pulley and the proximal phalanx were preserved, and all other soft and bony tissues were removed. A 1.5-mm (0.062-in) K-wire was inserted through the proximal phalanx, parallel to the long axis of the bone. Palmaris longus tendons were harvested from their insertion to their musculotendinous junction. As recommended clinically when extrasynovial tendons are used for tendon grafting, 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 2 pieces, proximal and distal, thus creating 32 PL segments, which were randomly assigned into 4 experimental groups, with 8 in each group.

Tendon surface modification
Lubricin was purified from bovine synovial fluid, as reported in a previous study, and preserved at −20°C until used. The purified lubricin was diluted with a solution of 0.1 mol/L 2-(N-morpholino) ethanesulfonic acid (MES) (Sigma, St. Louis, MO) and 0.15 mol/L NaCl to 260 μg/mL. The PL tendons were randomly assigned to 1 of 4 treatment groups: saline control, cd-HA gelatin, cd gelatin plus lubricin, and cd-HA gelatin plus lubricin (Table 1). In the cd gelatin plus lubricin and cd-HA gelatin plus lubricin groups, the tendons were first coated with cd gelatin or cd-HA gelatin without lubricin, then wrapped in a smooth rubber sheet for 10 minutes. Excess lubricant on the tendon surface was removed by gliding the tendon against the pulley for 5 cycles of simulated flexion and 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 the A2 pulley of the digit. 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, 2 custom-made tensile load transducers, and a mechanical pulley (Fig. 1). The load transducers were connected to the distal and proximal ends of the PL tendon. A 4.9-N load was connected to the distal transducer to maintain tension on the PL tendon. The proximal load transducer was connected to a custom-made mechanical actuator with a small linear slide that was driven by a precision
gearhead direct-current motor. Based on the experience of previous studies, 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 load at a rate of

**TABLE 1. Formulation of the Tendon Surface Treatments**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.9% NaCl, 0.1 mol/L MES (Sigma, St Louis, MO) at pH 6.0</td>
</tr>
<tr>
<td>cd-HA gelatin</td>
<td>1% HA (Acros, Geel, Belgium), 95%, 1.5 × 10⁶ molecular weight 10% gelatin (Sigma)</td>
</tr>
<tr>
<td></td>
<td>1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma)</td>
</tr>
<tr>
<td></td>
<td>1% N-hydroxysuccinimide (NHS) (Pierce, Rockford, IL)</td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl and 0.1 mol/L MES at pH 6.0</td>
</tr>
<tr>
<td>cd gelatin plus lubricin</td>
<td>10% gelatin, 1% EDC, 1% NHS, in 0.9% NaCl and 0.1 mol/L MES at pH 6.0</td>
</tr>
<tr>
<td></td>
<td>After 5 cycles of tendon motion, add 260 μg/mL bovine lubricin, 0.9% NaCl, 0.1 mol/L MES at pH 6.0</td>
</tr>
<tr>
<td>cd-HA gelatin plus lubricin</td>
<td>1% HA, 10% gelatin, 1% EDC, 1% NHS, in 0.9% NaCl and 0.1 mol/L MES at pH 6.0</td>
</tr>
<tr>
<td></td>
<td>After 5 cycles of tendon motion, add 260 μg/mL bovine lubricin, 0.9% NaCl, 0.1 mol/L MES at pH 6.0</td>
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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.
2 mm/second. The excursion distance was 19 mm, an average excursion of the human digital flexor tendon at the A2 pulley. 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 this calculation: (F2flexion—F2extension)/2.

The data were initially recorded in the normal PL tendon for 1 cycle. After tendon surface treatment, data were recorded after every 50 cycles up to 500 cycles and then after every 100 cycles up to 1000 cycles.

**Statistical analysis**

The gliding resistance before the surface treatment and after 1000 cycles of tendon motion was analyzed using 1-way analysis of variance. A Tukey-Kramer post hoc test for individual comparisons was used if there was a significant difference. A p < .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 4 groups (p = .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 plus lubricin, and cd-HA gelatin plus lubricin groups was 0.75 ± SD 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 plus lubricin, and cd-HA gelatin plus lubricin groups was significantly lower than that of the saline control after 1000 cycles (p < .05). The gliding resistance of the saline control PL tendons increased 190% over 1000 cycles of tendon motion (p < .05). There was no significant difference in gliding resistance before and after 1000 cycles for the cd-HA gelatin, cd gelatin plus lubricin, and cd-HA gelatin plus lubricin–treated groups (Fig. 2).

The trend of gliding resistance in each group is shown in Figure 3. The gliding resistance of the saline-treated control PL tendons 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 plus lubricin, and cd-HA gelatin plus lubricin groups decreased within the first 50 cycles and then increased gradually over the 1000 cycles. The rate of change in gliding resistance per cycle of motion (ie, the slope of the gliding resistance versus cycle number curve displayed in Fig. 3) from 100 to 1000 cycles was significantly lower for the cd-HA gelatin plus lubricin–treated tendons than for the saline control tendons (p < .05) (Fig. 4).

**DISCUSSION**

The human PL tendon is a common source for tendon graft clinically because the functional loss at the wrist donor site is slight, it is in the same surgical field as the flexor tendon, and it is easily accessible. The canine FL tendon is commonly used for tendon grafting experimentally because there is no true canine equivalent of the PL tendon, which is present only in primates. Although both the human PL and the canine FL tendons are extrasynovial and seem to be similar, there is a difference in the gliding resistance between the normal human PL and the canine FL tendon. In a previous study, the mean gliding resistance of the human PL tendon and the canine FL tendon were reported to be 0.52 ± 0.22 N and 0.09 ± 0.03 N, respectively. There also might 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 plus lubricin, and cd-HA gelatin plus lubricin groups was significantly lower (p < .05) than that of the saline control group 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 (p > .05), 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 might 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 the least with the cd-HA gelatin plus lubricin tendons, but this difference was significant only when compared to the untreated tendons. A larger sample size might have confirmed a statistically significant difference, but without in vivo data, it is difficult to determine whether 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 HA and lubricin might improve the gliding ability of a human graft tendon in vivo.

REFERENCES