Effect of hyaluronic acid on the excursion resistance of tendon grafts

A BIOMECHANICAL STUDY IN A CANINE MODEL IN VITRO


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The excursion resistance between the tendon and pulley is an important factor contributing to the limitation of function after surgery to the hand. The administration of hyaluronic acid (HA) in the early rehabilitation after tendon grafting may help to prevent adhesions. We evaluated changes in the excursion resistance between potential sources of flexor tendon grafts and the annular pulley in a canine model after administration of HA.

The intrasynovial and extrasynovial tendons were soaked in 10 mg/ml of HA for five minutes. The excursion resistance between these tendons and the annular pulley of an intact proximal phalanx and that of the same tendons of the opposite foot without administration of HA were evaluated. The tendon of flexor digitorum profundus of the second toe without administration of HA was used as a control.

The gliding resistance of canine tendons was significantly decreased after the administration of HA especially in the extrasynovial tendons. Our findings suggest that the administration of HA may improve the gliding function of a flexor tendon graft.

Primary repair of a flexor tendon is occasionally impossible and free tendon grafts using extrasynovial tendons of palmaris longus or plantaris are often used. However, adhesions between the grafted tendon and surrounding tissue resulting in restriction of movement and contracture of the finger joints are frequently seen. The degree of adhesion formation may be influenced by the regime for rehabilitation or the source of the donor. A reduction of the friction experienced by the graft may facilitate movement of the tendon.

Methods used to reduce the friction in the tendon-pulley unit include selection of the optimal source for donation of the graft and administration of a lubricant. We have already noted that intrasynovial tendons may be better sources for the graft and have considered the possibility of using hyaluronic acid (HA) as a lubricant. HA is a long polysaccharide chain consisting of repeating disaccharide units of N-acetyl-glucosamine and glucuronic acid, which occurs naturally in synovial fluid and gives it viscoelasticity.

We have examined the effect of the administration of HA on the excursion resistance of simulated tendon grafts with an intact annular pulley.

Materials and Methods

Preliminary examination to determine the soaking time. The excursion resistance between the annular pulley and the peroneal tendon of six dogs was measured to determine the optimum soaking time. Initially, the excursion resistance between each tendon and the annular pulley was measured as a control. The tendons were then soaked in 10 mg/ml of HA for one, five and ten minutes. The excursion resistance between the pulley and tendon was then measured. Tendons were washed with 1000 ml of saline solution for five minutes before each testing. The order of testing in each time group was random. The differences in the excursion resistance between groups including the control and at different angles were assessed by the Kruskal-Wallis test. Scheffe’s F-test was then used for a post-hoc comparison of individual means. The level of significance was set at p = 0.05. The excursion resistance of tendons tested for five minutes was significantly lower than in those tested for one minute at all angles (p < 0.05). However, there were no significant differences between the one-minute group and control, and between the five- and ten-minute groups. The soaking time for tendon graft models used with HA was decreed to be five minutes.

Models of tendon graft and administration of HA. We used the hindpaws of six adult mixed-breed dogs weighing between 15 and 30 kg. The animals were killed by the administration of intravenous pentobarbital following the


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guidelines set by our Institutional Animal Care and Use Committee. The fresh-frozen second toe of a hindpaw was used for each in vitro model. All specimens were thawed immediately before testing and kept moist in a saline bath throughout the testing procedure. The room temperature was kept at 21˚C during the study.

The tendons used were the ipsilateral tendon of peroneus proximal to the peroneal retinaculum (P group), the ipsilateral tendon of extensor digitorum communis, the ipsilateral tendon of flexor superficialis of the third toe (S group) and the tendon of flexor digitorum profundus to the second toe (C group). Because the proximal part of extensor digitorum communis passes beneath the extensor retinaculum and may therefore be considered intrasynovial, this tendon was tested twice, once for its distal extrasynovial portion (E group) and once for its proximal subretinacular portion (R group). These two segments were tested by using both a distal-proximal and proximal-distal orientation of extensor digitorum communis. In the normal proximal-distal orientation, the subretinacular portion of the tendon remained proximal to the tendon sheath throughout testing. In the reversed distal-proximal orientation, the subretinacular portion of the tendon remained within the tendon sheath throughout. Each graft was tested once, except for the tendon of extensor digitorum communis which was tested once in each of two orientations. The excursion resistance between these tendons and an intact annular pulley of the hindpaw, which corresponds to the A2 pulley in man was evaluated. The excursion resistance of the same tendons of the opposite flexor digitorum profundus of the second toe (C group) was measured as a control.

Measurement of the interaction between the tendon and pulley. The concept of measurement of friction and its application to the tendon-pulley unit have previously been verified and validated.8,9

A tendon sliding through a curved pulley is analogous to a cable wrapped around a fixed mechanical pulley. The tensions in the cable are F1 and F2 at each end, respectively. If the movement of the cable is from transducer F1 to transducer F2, then the force at F2 is greater than that at F1 because of the friction f, and f = F2-F1. This model is the basis for our experiment.

A transverse incision was made through the synovial sheath just distal to the annular pulley with the digit in full extension to mark the lateral surface of the tendon of flexor digitorum profundus. The tendon was then pulled proximally until full flexion was achieved at the proximal and distal interphalangeal joints. In this position, the tendon was again marked through the previous incision. The distance between these two marks indicated the area of physiological excursion.

To exclude the influence of interaction between tendon and bone, the distal end of the proximal phalanx was divided at the distal edge of the pulley, and the plantar cortex of the proximal phalanx removed. A 1.5 mm Kirschner wire was inserted through the proximal phalanx parallel to the longitudinal axis of the bone to support the specimen during the experiment.

Each specimen was then mounted on the testing device. The plantar aspect of the specimen faced upwards and the proximal aspect was towards the device actuator. The measurement system consisted of one custom-built mech-
anical actuator with a linear potentiometer and two custom-made tensile load transducers (Fig. 1).

These transducers were connected to the proximal and distal ends of the tendon using dacron cord. The proximal transducer (F2) was connected to the mechanical actuator and the distal transducer (F1) to the weight (250 g). The actuator was positioned at a preselected angle ($\alpha$) which was defined as the angle in degrees formed between the horizontal plane and the proximal cable extension. The mechanical pulley between the load and the distal load transducer was positioned at a preselected angle ($\beta$) which was defined as the angle formed between the horizontal plane and the extension of the distal cable. The sum of the angles $\alpha$ and $\beta$ was considered as the angle of the arc of contact. The tendon was pulled proximally at a rate of 2.0 mm/s by the actuator and opposed by the weight. Movement of the tendon towards the actuator was regarded as flexion. F1 and F2 and the corresponding excursion were recorded by a digital computer at a sampling rate of 10 Hz. The excursion of each tendon was limited to the measured distance of the physiological area of the control as described above. The angles $\alpha$ and $\beta$ were varied to five different positions. Three trials were performed for each of the five positions as follows: $\alpha$, $\beta$ = 15˚, 5˚; 20˚, 10˚; 30˚, 10˚; 30˚, 20˚ and 30˚, 30˚. These positions and the testing rate were selected because they were comparable to those used in previous studies.4-6,9

The order of testing the grafts within each toe was allocated randomly.  

**Statistical analysis.** The plots of the measurements of F1 and F2 versus excursion were examined for each trial. Since the trials were generally identical and the first run was considered to be a trial, the mean of the last two runs of flexion was selected for analysis for each angle. The mean force differences of F2 and F1 for the whole excursion were obtained and regarded as the resistance at the interface between the tendon and pulley for the given arc of contact. The tendons were classified as intrasynovial (S, R and C groups) and extrasynovial (E and P groups). The groups without administration of HA were compared with each other statistically. Each tendon which received HA was compared with its fellow which did not.

The differences in the excursion resistance between tendons with and without administration of HA at different angles were assessed by the Kruskal-Wallis test. Scheffe’s F-test was then used for a post-hoc comparison of individual means. Significant differences in the excursion resistance between each tendon with and without administration of HA were assessed by the Wilcoxon matched-pairs test. The level of significance was set at $p = 0.05$.

**Results**

The mean and SD of the excursion resistance of all groups are summarised in Tables I and II.

### Table I. The mean (SD) gliding resistance between the tendon and pulley without HA in the various groups in Newtons

<table>
<thead>
<tr>
<th>Group</th>
<th>Angle (˚)</th>
<th>Control</th>
<th>S</th>
<th>R</th>
<th>E</th>
<th>P</th>
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<tbody>
<tr>
<td>20</td>
<td>0.10 (0.04)</td>
<td>0.10 (0.03)</td>
<td>0.11 (0.01)</td>
<td>0.13 (0.04)</td>
<td>0.15 (0.04)</td>
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<tr>
<td>30</td>
<td>0.11 (0.03)</td>
<td>0.11 (0.03)</td>
<td>0.13 (0.02)</td>
<td>0.19 (0.04)</td>
<td>0.19 (0.02)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.13 (0.04)</td>
<td>0.14 (0.05)</td>
<td>0.17 (0.03)</td>
<td>0.22 (0.03)</td>
<td>0.21 (0.03)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.15 (0.04)</td>
<td>0.17 (0.04)</td>
<td>0.19 (0.04)</td>
<td>0.25 (0.04)</td>
<td>0.25 (0.04)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.21 (0.03)</td>
<td>0.21 (0.06)</td>
<td>0.25 (0.05)</td>
<td>0.32 (0.03)</td>
<td>0.29 (0.04)</td>
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</tr>
</tbody>
</table>

### Table II. The mean (SD) gliding resistance between the tendon and pulley with HA in all groups in Newtons

<table>
<thead>
<tr>
<th>Group</th>
<th>Angle (˚)</th>
<th>Control</th>
<th>H</th>
<th>SH</th>
<th>RH</th>
<th>EH</th>
<th>PH</th>
</tr>
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<tr>
<td>20</td>
<td>0.08 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.06 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.09 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.09 (0.02)</td>
<td>0.13 (0.03)</td>
<td>0.08 (0.02)</td>
<td>0.13 (0.02)</td>
<td>0.10 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.10 (0.02)</td>
<td>0.14 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.14 (0.03)</td>
<td>0.11 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.13 (0.02)</td>
<td>0.16 (0.02)</td>
<td>0.12 (0.02)</td>
<td>0.15 (0.03)</td>
<td>0.14 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.15 (0.03)</td>
<td>0.18 (0.02)</td>
<td>0.15 (0.03)</td>
<td>0.19 (0.04)</td>
<td>0.18 (0.03)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The excision resistance between the tendon and pulley without administration of HA was shown in Figure 2. No significant difference was seen in the intrasynovial tendons including the control. The excursion resistance was significantly higher in both extrasynovial tendons (groups P and E) as compared with the control at all angles except at 20˚: at 30˚, $p < 0.001$ between control and both P and E groups; at 40˚ $p = 0.0017$ between the control and E group, $p = 0.0093$ between the control and P group; at 50˚, $p = 0.0016$ between the control and E group and $p = 0.0012$ between the control and P group; at 60˚, $p = 0.0064$ between the control and E group and $p = 0.0456$ between the control and P group. The difference in the excursion resistance between the E and P groups was not significant. Extrasynovial tendon groups produced an
excursion resistance higher than that of intrasynovial groups. Between the S and P groups, the difference in the excursion resistance was significant at 30° and 50°; at 30°, p = 0.0018, at 50°, p = 0.0141. Between the S and E groups, the difference in the excursion resistance was significant at all angles except at 20°: at 30°, p = 0.005; at 40°, p = 0.0320; at 50°, p = 0.0180 and at 60°, p = 0.0141. Between the R and E groups, the difference in the excursion resistance was significant only at 30° (p = 0.0081).

The excursion resistance between the tendon and pulley after the administration of HA. The results for the extrasynovial tendon groups with and without HA and the control are shown in Figure 3. The differences in the excursion resistance between the distal extrasynovial portion of the tendon of extensor digitorum communis with HA (EH group) and without HA (E group) were significant at all angles except at 20° (at 30, 40, 50 and 60°; p = 0.027). The difference in the excursion resistance between the peroneus tendons with HA (PH group) and without HA (P group) was significant at all angles (at 20, 30, 40, 50 and 60°; p = 0.0277). The results for intrasynovial tendon groups with and without HA are shown in Figure 4. No significant difference was seen in these tendons except between the R and RH groups. The differences in the excursion resistance between the R and RH groups were significant at all angles (p = 0.0277 at 20, 30, 40 and 50°; at 60° p = 0.0467).

Discussion
Grafts are often required in managing injuries to flexor tendons and it is well recognised that digital function after grafting is worse than that after primary repair. The kinematics of tendon gliding are extremely sophisticated and restoration of gliding is influenced by many factors. Those which may influence the final clinical outcome of a tendon graft include the site and size of the defect, the suture material used, the suture technique, the status of the surrounding soft tissue, the integrity of the pulley, capsular stiffness, the size of the graft, the mechanical properties of the graft source, the timing of the procedure and the rehabilitation programme. Another potential factor, which may influence the outcome, is the lubricant. An ideal reconstruction of a flexor tendon injury is one which is reinforced by the administration of a lubricant which does not interfere with the healing of the tendon but will allow low-resistance gliding.

We have developed a technique to measure one component of tendon gliding, namely the frictional resistance between a tendon and the pulley. This technique has the advantage of measuring the tendon-pulley interaction.
directly and we have observed significant differences in the excursion resistance between tendon grafts with and without administration of HA for extrasynovial tendon donor sources. While the excursion of extrasynovial grafts was significantly higher than that of the intrasynovial graft without administration of HA, it was approximately the same as that of the normal tendon of flexor digitorum profundus after the administration of HA. Although this was only an in vitro study, we believe that a clinical advantage may be obtained in rehabilitation after tendon grafting utilising HA in vivo.

Our study obtained pilot data as to the efficacy in vitro of the administration of HA in order to plan an in vivo study. The value of our study lies in the ability to measure directly the efficacy of HA in the interaction of the pulley and the tendon. However, an in vitro model was used and the excursion resistance was measured only between a single annular pulley and tendon. The rate of movement of the tendon was not physiological, the long-term effects of HA were not addressed, the sample size was not large, and a dose-titration evaluation was not performed.

There are many reports which describe the effects of HA in vitro and in vivo. The formation of scars and granulation tissue is suppressed by HA,14 which also prevents the formation of adhesions after repair of the tendon without interfering with healing.15 The decrease in the formation of adhesions after treatment with HA may be the result of a decrease in the formation of new extracellular matrix due to the inhibition of mononuclear phagocytes and lymphocytes.16 HA suppresses the release of fibronectin which plays a crucial role in the adhesion, proliferation and differentiation of various cells.17 It also regulates the activity of polymorphonuclear leucocytes.18 Although the mechanism of action of HA as a lubricant in the tendon-tendon sheath unit is not well known, in this study the excursion resistance between the extrasynovial tendon and the annular pulley decreased significantly after the administration of HA. The function of HA is not only as a lubricant but also in preventing adhesion and the extrasynovial tendon graft with administration of HA may simulate the intrasynovial tendon more closely.

The goals of reconstruction after injury to the flexor tendon are the restoration of grip strength and active digital movement. Most articles concerned with tendon grafts focus on problems of the healing and adhesion of tendons.3,11,19 The friction at the repaired tendon-pulley interface in vitro has also been studied and this interaction has been quantified.20,21 We have reported the results of direct measurements of friction between the grafted tendon and...
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One of the factors affecting friction between the grafted tendon and pulley is the source of the graft. Intrasynovial tendon grafts produce less excursion resistance than extrasynovial grafts.4,6

While the problem of adhesions after repair of the flexor tendon has been discussed widely,3,10,12,15,23,24 most authors have emphasized the role of the surgical technique and early mobilisation in the prevention of the detrimental effects of adhesions. We believe that an advantage may be expected from the administration of HA, not only in the early rehabilitation after tendon grafting to prevent adhesions, but also to decrease excursion resistance.

Several recent articles have studied the difference between intrasynovial and extrasynovial tendon grafts. Intrasynovial grafts show significant differences from extrasynovial in both surface morphology and vascularity.25 They heal with less cellular necrosis and with less extensive adhesions and they survive better than extrasynovial grafts.26,27 They also integrate with less scar formation between the tendon surface and surrounding tissues.28 The synthesis of proteoglycan matrix proteins and DNA is also different between these two types of tendon surface29 as are the mechanisms of surface lubrication. The advantages of the intrasynovial tendon graft are better healing, less adhesion and less excursion resistance.

The results of biomechanical testing of the tendon-pulley interaction after tendon grafting in a canine model in vitro revealed friction with administration of HA. However, in vitro models cannot address the complex effects of the surgery itself, wound healing, structural changes in the reconstructed tendon, and the relationship between in vitro friction and the friction which is present after wound healing has occurred.

The results of our study suggest that the administration of HA may improve the outcome of flexor tendon grafting. An in vivo study of an animal model to test excursion resistance between the pulley and grafted tendon after administration of HA is now required.

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References


