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The Effects of Hylan G-F 20 Surface Modification on Gliding of Extrasynovial Canine Tendon Grafts *In Vitro*

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Abstract

Purpose—Studies have shown that a lubricant exogenously applied on extrasynovial tendon surfaces can reduce the gliding resistance after flexor tendon repair; however, the reagents that have been tested are solely for experimental testing and are not available for clinical use. The purpose of this study was to investigate the effect of exogenously applied hylan G-F 20, a U.S. Food and Drug Administration–approved hyaluronic acid for the treatment of osteoarthritis, on extrasynovial tendon gliding resistance in an *in vitro* canine model.

Methods—Twenty-four canine peroneus longus (PL) tendons and proximal pulleys of the ipsilateral paws were treated with 1 of 3 solutions: saline, carbodiimide derivatized hylan G-F 20, or unmodified hylan G-F 20. The gliding resistance of each tendon preparation was then measured over 1000 cycles in a saline bath.

Results—After 1,000 cycles, the gliding resistance of the PL tendons treated with unmodified hylan G-F 20 decreased significantly compared with the saline-treated tendons. The gliding resistance of the PL tendons treated with modified hylan G-F 20 increased significantly compared with the saline group.

Conclusions—The PL tendons treated with pure hylan G-F 20 showed a positive effect on the gliding resistance.

Clinical relevance—The results of this *in vitro* canine study suggest that exogenously applied hylan G-F 20 improves gliding of the extrasynovial tendon graft. This material may be capable of reducing friction over flexor tendon repair sites and flexor tendon grafts.

Keywords

Adhesions; hyaluronic acid; tendon injury; tendon repair

Flexor tendon injuries are common, especially in the young and working-age population. Although the results of flexor tendon repair have improved in recent decades, successful outcome, particularly in zone 2, is still difficult to achieve. In complicated injuries, tendon grafts play an important role in reconstructing severely scarred or missing tendons. Extrasynovial tendon grafts are used most frequently because they are easily harvested without producing serious morbidity at the donor site; however, the main disadvantage of these grafts is a higher gliding resistance compared with intrasynovial tendons, and this in turn can lead to adhesions. [1–4] Previous studies have shown that tendon gliding capability is

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an important factor influencing the outcome of tendon repair and that improved gliding results in fewer adhesions.[5,6]

In an effort to improve tendon gliding, various exogenous agents have been applied to tendon grafts to decrease friction. One such agent is hyaluronic acid (HA). HA is secreted from intrasynovial tendons by synovial cells and serves as a lubricant between the intrasynovial tendon and the pulley.[7] HA exogenously applied onto extrasynovial tendon grafts alone has been shown to be insufficient because it is diluted within the body and quickly eliminated during tendon motion.[8] Momose et al[9] reported a chemically modified carbodiimide derivatized HA (cd-HA) that can bind to the extrasynovial tendon, decreases gliding resistance, and resists degradation caused by motion. Several additional studies have demonstrated that combining this cd-HA with gelatin or lubricin resulted in an even greater reduction in gliding resistance, decrease of adhesion formation, and improving digital function in both *in vitro* and *in vivo* animal models.[10–15]

Unfortunately, these reagents have been tested solely in the experimental laboratory setting and are not pharmaceutically available for clinical use. The purpose of this study was to investigate whether clinically available HA, already U.S. Food and Drug Administration (FDA)–approved for the treatment of other medical conditions, could produce improvements similar to those seen in an experimental animal model. In this study, we examined the effects of hylan G-F 20 (Synvisc, Genzyme Corporation, Cambridge, MA) on the gliding resistance of extrasynovial tendons compared with a control group (treated with saline solution) and to tendons treated with a carbodiimide derivatized hylan G-F 20 group (cd-hylan G-F 20). Hylan G-F-20 is FDA-approved for the intra-articular treatment of osteoarthritis of the knee. It is an elastoviscous fluid of high molecular weight containing chemically cross-linked hylan A and hylan B polymers, derivatives of hyaluronan (sodium hyaluronate), produced from chicken combs.

If hylan G-F 20 can reduce gliding resistance within this *in vitro* experiment, it could then be applied within the clinical setting to reduce tendon friction during the early postoperative period and potentially improve the results of flexor tendon repair and flexor tendon grafting.

MATERIALS AND METHODS

The study design is similar to previously published work.[11] Twenty-four hind legs were harvested from 12 mongrel dogs that were already being killed for other projects with Institutional Animal Care and Use Committee approval. We preserved the paws in the freezer at −20°C after harvest. Paws were then thawed at room temperature immediately before use. From each hind leg, we dissected the peroneus longus (PL) tendon for use as the extrasynovial tendon graft. We removed the paratenon, with care to prevent damage of the tendon surface, as recommended when using extrasynovial tendon grafts clinically.[16,17] We also dissected the ipsilateral second digit from which the proximal and middle phalanges and the proximal pulley (which is similar to the human A2 pulley) were used. The flexor digitorum profundus was removed; the flexor digitorum superficialis tendon with its insertion was preserved. A Kirschner wire was drilled through the proximal and middle phalanges to fix the proximal interphalangeal joint in full extension.

The PL tendons along with their corresponding digits were randomly assigned to 3 groups of 8 tendons each. The first group was a control group in which the tendons were simply immersed in 0.9% sodium chloride solution before testing (saline group). In the second and third groups, tendons were treated with either cdhylan G-F 20 (cd-hylan G-F 20 group) or an unmodified hylan G-F 20 (hylan G-F 20 group).

In order to chemically bind the hylan G-F 20 to form cd-hylan G-F 20, we used the same method to produce cd-HA as described by Momose et al.[9] Ninety percent hylan G-F 20 was mixed with 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 1% N-hydroxysuccinimide (NHS) and 8% saline (0.9% NaCl, pH 6.0). The hylan G-F 20 group was treated with 100% unmodified hylan G-F 20. Tendons were coated by immersion in 1 of the previously described solutions for 1 minute. Afterward, tendons were placed in a sealed silicon tube for 30 minutes to cure them[18] and to keep them hydrated until friction testing. Excess gel on the surface of the tendon was removed by rubbing a gloved finger against the tendon surface several times before the treated tendon was inserted under the proximal pulley of the digit. Gliding resistance between the tendons and the proximal pulley was measured using a custom-made mechanical test system previously described (Fig. 1).[19–21] The digit, with the PL graft inserted through the A2 pulley, was mounted to the device in a room temperature saline bath with the volar side of the pulley facing upward. Custom-made tensile load transducers were attached on each end of the tendon. One transducer (F1) was connected to the distal tendon end and a 4.9-N weight. The other transducer (F2) was attached to the proximal tendon end and a mechanical actuator. The transducers and actuator were oriented to create a 50° arc of contact (30° on the proximal end and 20° on the distal end). Both the weight and the arc of contact were selected based on previous studies.[4,6,21,22] The actuator pulled the tendon proximally (flexion) and then reversed direction (extension) at a velocity of 2 mm/sec and with an excursion distance of 14 mm, which is the average canine digital flexor tendon excursion.[9] Transducer data were recorded for the first cycle, every 10 cycles up to 200 cycles, then every 50 cycles up to 500 cycles, and finally every 100 cycles up to the maximum of 1000 cycles. Gliding resistance between the PL and the A2 pulley was calculated by determining the mean difference between the F2 and the F1 transducers.[4] Gliding resistance was expressed as mean ± SD in Newtons (N), and data were analyzed by 1-way analysis of variance. A post-hoc Tukey test was used for individual comparisons. P less than .05 were considered to be significant.

RESULTS

As observed in previous studies, the gliding resistance of the PL tendons treated with saline increased rapidly in the first 200 cycles, eventually reaching a plateau around 0.8 N to 0.9 N. After 1000 cycles, these tendons reached a gliding resistance of 0.93 ± 0.17 N (Fig. 2). Gliding resistance of the PL tendons treated with cdhylan G-F 20 was initially (during cycle 1) significantly lower (0.11 ± 0.04 N) than the saline group (P = .019). However, gliding resistance of these tendons increased more rapidly than the saline group in the first 200 cycles to 0.94 ± 0.22 N, and even after 1000 cycles, the increase appeared to continue, having risen to a gliding resistance of 1.40 ± 0.24 N, significantly (P = .001) higher than the saline group (0.93 ± 0.17 N) (Fig. 3).

Tendon treatment with unmodified hylan G-F 20 resulted in an initial gliding resistance of 0.07 ± 0.01 N and increased more slowly and consistently compared with the other groups. After 200 cycles, gliding resistance had increased to 0.28 ± 0.17 N and progressed to 0.59 ± 0.23 N after 1000 cycles. This final gliding resistance was significantly lower than the saline group (P = .013).

DISCUSSION

Flexor tendon adhesions remain a complication of flexor tendon repair and tendon grafting. Several advances have been made to prevent adhesion formation, including improved surgical techniques, new suture material, and the addition of various pharmaceutical agents in an effort to inhibit scarring or lower friction along the tendon surface. Increased friction at the repair site has been linked to gap and adhesion formation.[23]

Friction in a normal intrasynovial tendon is low and tends to remain low with repeated tendon excursion, This is caused by the smooth surface of the tendon and opposing sheath as well as the presence of lubricin and HA within the tendon sheath.[24,25] Extrasynovial tendon grafts have rougher surfaces that result in a higher gliding resistance and generate more adhesions when they are used for intrasynovial grafting.[1–4,26] The findings of this study suggest that clinically available HA is capable of lowering the gliding resistance on extrasynovial grafts and could potentially reduce adhesion formation.

Taguchi et al[11] reported, in a similar study design, the effects on gliding resistance of extrasynovial tendon grafts treated with both cd-HA combined with gelatin and a chemically modified gelatin combined with lubricin. After 1000 cycles, the gliding resistance resulting from these treatments were found to be 0.21 ± 0.07 N and 0.11 ± 0.01 N, respectively, results which are even lower than the gliding resistance of the hylan G-F 20 group in our present study. Unfortunately, we were not able to test gelatin and lubricin in this study because, currently, there are no suitable FDA-approved gelatin or lubricin products available.

Compared with the results of previous studies investigating the effect of cd-HA,[9] the results of this study were quite different. Much higher friction was observed in the tendons treated with cd-hylan G-F 20 than in both the control group and the unmodified hylan G-F 20 group. We think this result might have been caused by the process used by the manufacturer to cross-link the HA to produce hylan G-F 20. It is our speculation that combining hylan G-F 20 with the EDC and NHS bound the amine and carboxyl groups too much, which caused crystallization. Another explanation could be that the high viscosity of hylan G-F 20 inhibited proper mixing with the EDC and NHS. The EDC and NHS could become more highly concentrated in some areas of the solution, which also might stimulate crystallization. Therefore, we collected saline solution from the water bath after friction testing and placed several drops onto regular histology glass for further analysis. Such crystallization of the solution was identified through light microscopy, as seen in Figure 4. These small, crystal-like particles might damage the tendon or pulley surface during repeated tendon motion against the pulley, leading to high gliding resistance owing to rough surfaces.

Although Momose et al showed that unmodified HA does not work as a lubricant to decrease the gliding resistance, our study showed that the gliding resistance of the tendon with unmodified hylan G-F 20 decreases from a mean of 0.93 N to a mean of 0.59 N.[9] It is likely that these findings can be explained by the cross linked HA in hylan G-F 20 and its viscosity, which may already have the same effect as our chemically modified HA (cd-HA); however, it is possible that combining hylan G-F 20 with a gelatin could decrease the gliding resistance even more, as reported by Taguchi et al.[11] Further investigation is warranted to confirm this.

Previous reports have described a decrease of tendon adhesions after the use of HA in vivo.[8,27–32] These findings might suggest that hylan G-F 20, when used as a surface treatment for extrasynovial flexor tendon grafts, may provide a beneficial mechanical and biological enhancement to improve tendon repair *in vivo*. It may even prevent adhesions in other kinds of flexor tendon repair surgery after injury. Another potential advantage of using hylan G-F 20 may be an extended half-life compared with that of normal liquid HA. Recently, Larsen et al[33] reported the half-life of the liquid HA was 1.5 days and the half-life of a hylan gel (similar to hylan G-F 20) was 8.8 days following intra-articular injection into rabbits. This suggests that the half-life of hylan G-F 20 in flexor tendon grafting might be longer as well, resulting in a longer effect compared with unmodified HA.

Clinically, it may be possible to inject HA within the flexor sheath during the postoperative period in those patients at risk for adhesion or with noteworthy edema in the flexor sheath.[34] The potential benefit of currently available HA solutions include the possibility of multiple injections over the course of rehabilitation and the ability to visualize the location of the injection with ultrasound. Further studies would be required to show clinical efficacy over standard early motion therapy protocols and to show that HA could be effectively injected within the flexor sheath.

Complications can arise from the use of high–molecular-weight HA products. Some studies reported local adverse reaction at the injection site when hylan G-F 20 was used for knee arthritis.[35,36] These events were reported to be "mild-to-moderate in nature, resolve spontaneously or after treatment of symptoms," and did not result in long-term complications. Further clinical investigation is warranted to rule out possible adverse reactions of hylan G-F 20 in flexor tendon grafts.

This study has several limitations. First, we did not know the details of the components in hylan G-F 20, such as the cross-linking process of the HA during manufacturing; therefore, we did not know in advance if this would influence our method to produce cd-hylan G-F 20. Second, we did not test tendon gliding resistance before treatment. There may be a difference in the initial gliding resistance between PL tendons of the tested groups; however, previous studies have not shown a variation of the initial PL gliding resistance.[9,11] If there was a difference, we would not expect that this alone could explain the significant difference in gliding resistance between our groups. Third, it is not clear if there was any residual hylan G-F 20 on the tendon surface after testing because we did not look for evidence of this using any method. Perhaps this may have provided us with a better understanding of the adhesive characteristics between the tendon surface and hylan G-F 20. Lastly, we tested hylan G-F 20 in an *in vitro* study. Further studies should be performed to determine the effects of hylan G-F 20 in an *in vivo* model.

The key finding in our study was a significant decrease of gliding resistance with the use of the FDA-approved and pharmaceutically available hylan G-F 20 on extrasynovial tendon grafts. In the future, hylan G-F 20 may be useful in the treatment of extrasynovial flexor tendon grafting and may even be useful in other flexor tendon repairs; however, *in vivo* studies should determine the safety of hylan G-F 20 in flexor tendon surgery and investigate the effects of combining hylan G-F 20 with a gelatin to decrease the gliding resistance to even lower levels.

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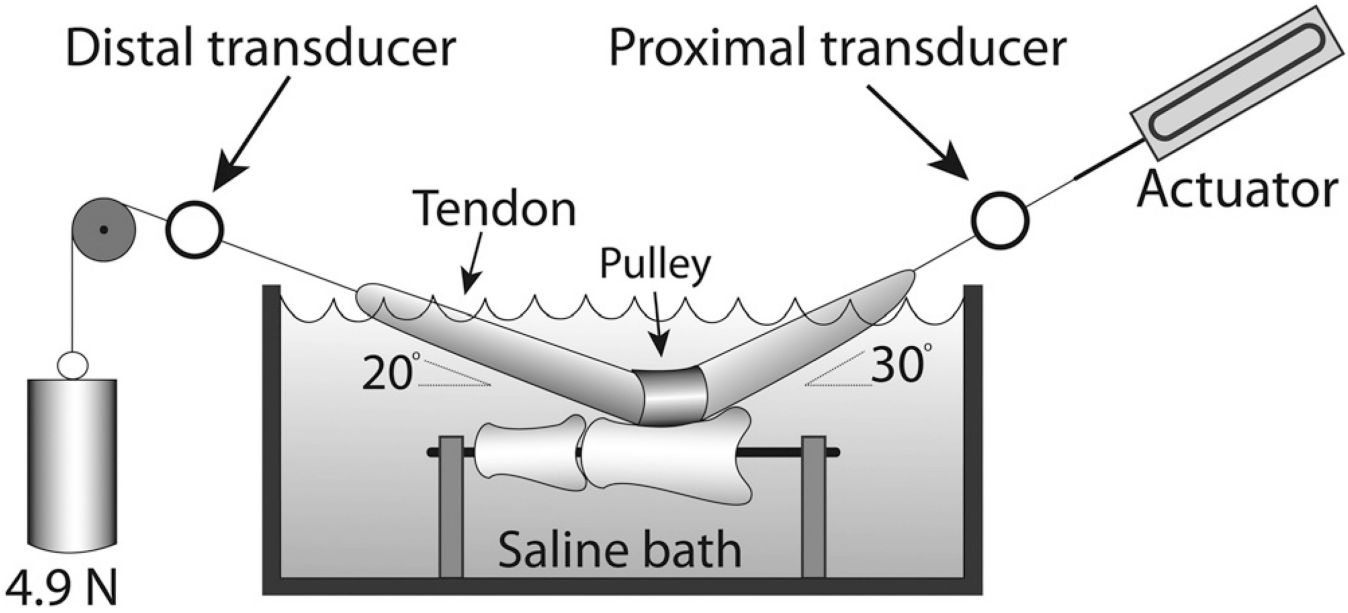

FIGURE 1.
Test device used for the measurements of the gliding resistance.

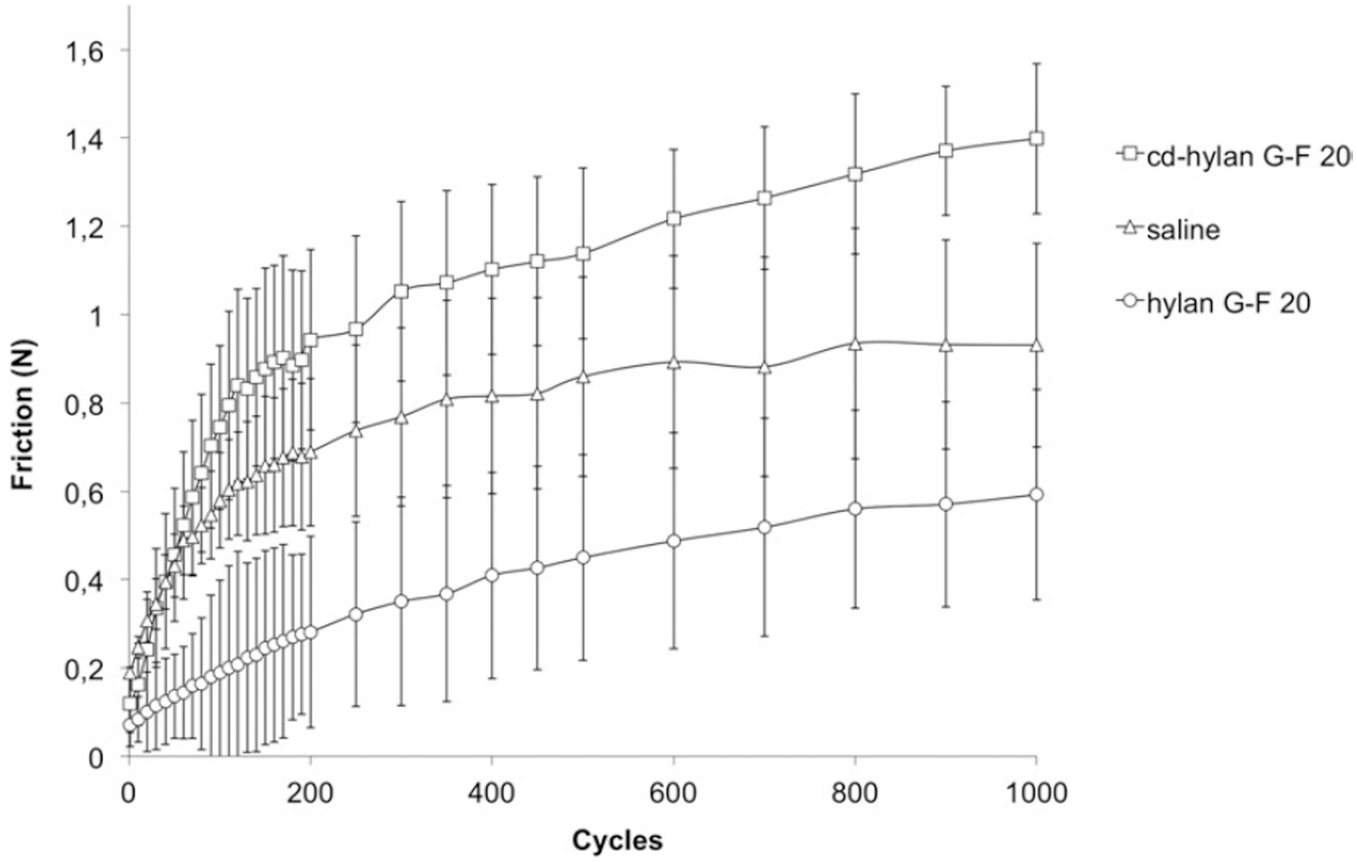

FIGURE 2.
Gliding resistance of the peroneus longus tendon in the 3 groups. Error bars represent SD.

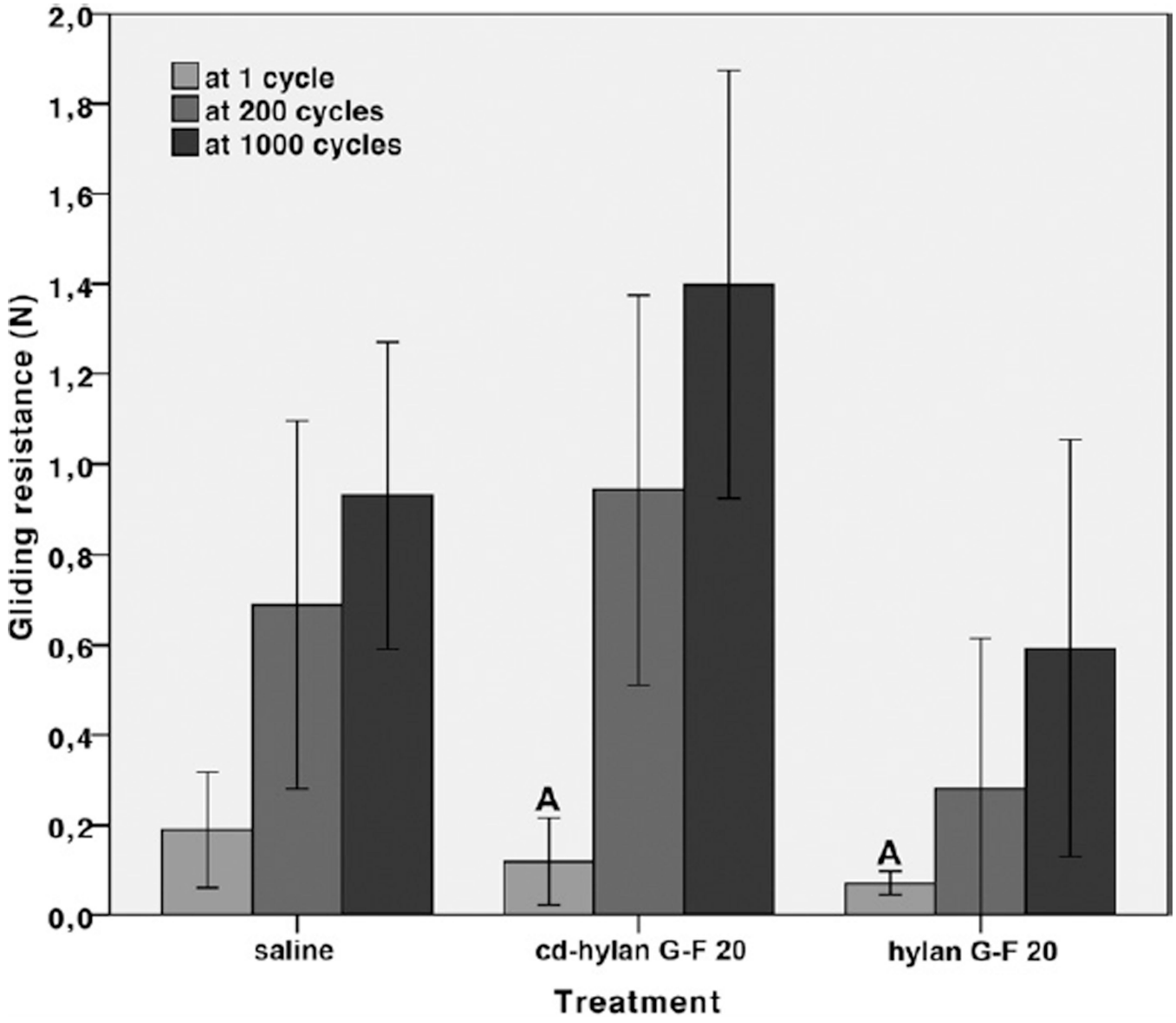

FIGURE 3.
Gliding resistance of the peroneus longus tendon. Error bars represent SD. The bars with a letter A were not significantly different from each other; all other groups had a significant difference.

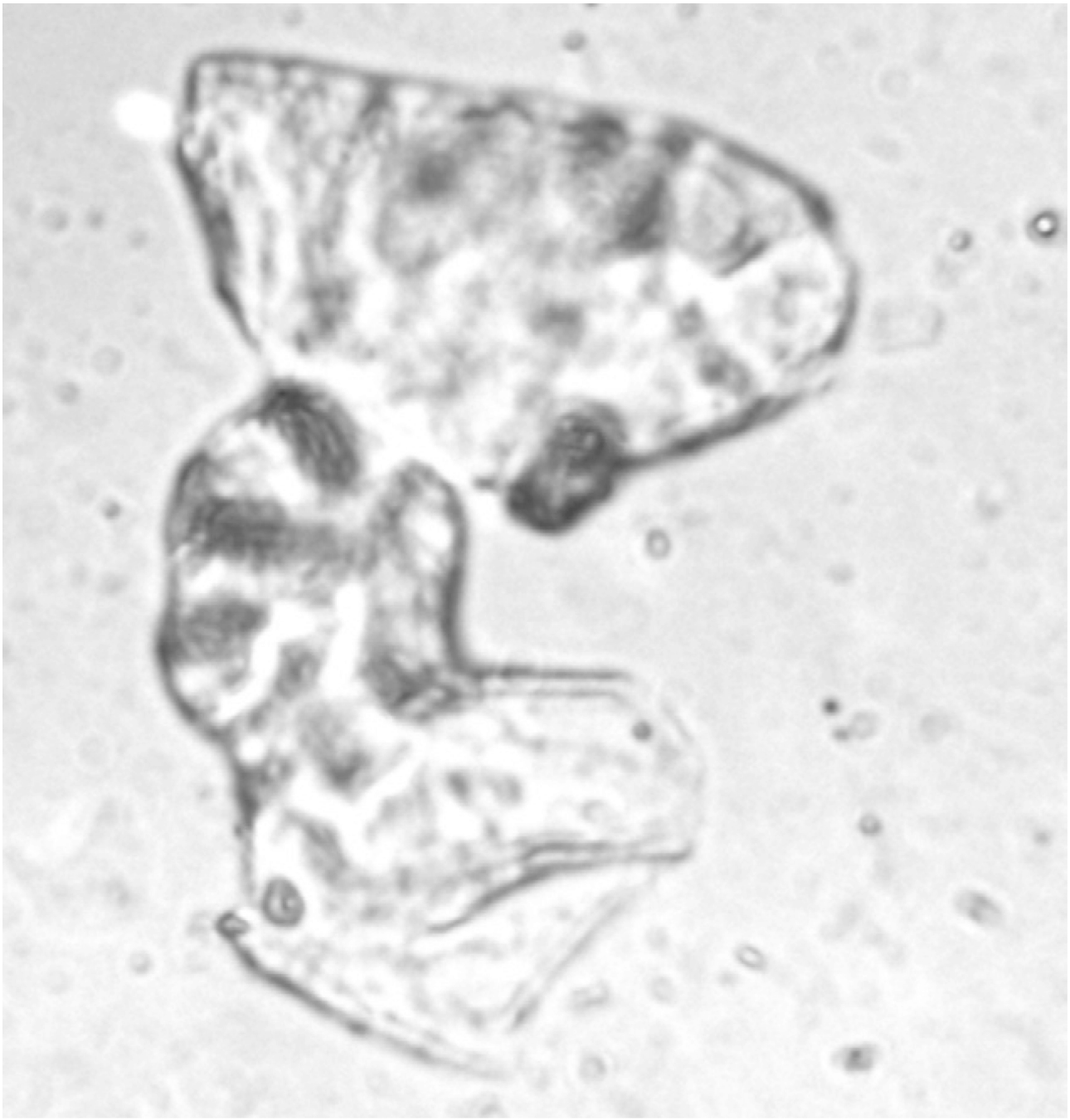

FIGURE 4.
Microscopic picture of the crystallization founded in the cd-hylan G-F 20 solution. These crystals were not present in unmodified hylan G-F 20.