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Application of Carbodiimide Derivatized Synovial Fluid to Enhance Extrasynovial Tendon Gliding Ability

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Abstract

Purpose—To investigate the effects of surface modification of extrasynovial tendon with a carbodiimide derivatized synovial fluid (SF) on the gliding ability of extrasynovial tendon for a possible tendon graft application.

Methods—We used 63 peroneus longus tendons from canine hind legs. We immediately assessed 3 tendons morphologically using a scanning electron microscope (SEM); these served as the normal tendon group. The other 60 tendons were randomly assigned to each of 6 experimental groups treated with (1) control (saline); (2) 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) plus 1% *N*-hydroxysuccinimide (NHS) (cd only); (3) 1% EDC/NHS plus 10% gelatin (cd-G); (4) SF plus 1% EDC/NHS plus 10% gelatin (cd-SF-G); (5) SF only; or (6) SF plus 1% EDC/NHS (cd-SF). We measured the gliding resistance for 1,000 cycles of simulated flexion-extension motion. We also observed the tendon surface smoothness by SEM.

Results—Compared with the first cycle in each group, the gliding resistance after 1,000 cycles of tendon motion was significantly increased in the control, cd only, cd-gelatin, SF only, and cd-SF groups ($p<.05$). In contrast, we found no significant difference in gliding resistance between the first cycle and 1,000 cycles for the cd-SF-G–treated group. In addition, the gliding resistance in the cd-SF, cd-G, and cd-SF-G groups was significantly lower than the control group after 1,000 cycles of tendon motion ($p<.05$) and the gliding resistance of the cd-SF-G group was significantly lower than both the cd-G and cd-SF groups ($p<.05$). On SEM, the surface treated with cd-SF-G was smooth after 1,000 cycles, whereas the other surfaces were rough.

Conclusions—Surface modification of extrasynovial tendon with cd-SF-G improves tendon gliding ability. This treatment may be useful clinically in improving the outcomes of tendon autografts.

Keywords

Extrasynovial tendon; flexor tendon; gliding resistance; graft; surface modification

Injuries to the flexor tendons in the digits are common and usually result in considerable disability. Although immediate primary repair after injury has been generally accepted and outcomes have been improved with postoperative controlled mobilization programs,[1–4] functional outcomes are occasionally poor. In the most complex cases, tendon grafts have an

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important role in salvage.[5] However, clinical outcomes after tendon autograft are generally poor owing to complications such as adhesion formation, which impairs hand function. Although the most complex injuries occur to intrasynovial tendons, usually tendon grafts are obtained from extrasynovial tendons because extrasynovial tendons are easily harvested without major ramification to the donor site[6–8] and intrasynovial sources are not readily available. However, experimental studies have shown that extrasynovial tendon grafts are associated with more adhesions to the surrounding tissues than are intrasynovial tendon grafts[9,10] and grafts harvested from intrasynovial tendons have better outcomes than extrasynovial grafts.[11–13] In addition, the gliding resistance of extrasynovial tendon is greater than that of intrasynovial tendon[14,15] and the surface of the extrasynovial tendon is rougher than that of intrasynovial tendon.[14,16]

Previous studies have also shown that higher gliding resistance results in greater adhesion formation[17] and extrasynovial tendon has a higher gliding resistance than intrasynovial tendon.[14] Tendon gliding resistance is an important factor influencing the outcome of tendon repair. To improve its gliding ability, surface modification of the tendon has been studied using surface lubricants such as hyaluronic acid, lubricin, and phospholipids.[15,18–20] Treating the extrasynovial tendon surface with a carbodiimide derivatized hyaluronic acid and gelatin mixture has been shown to improve tendon gliding ability *in vitro*[15,19,21] and improve digit function *in vivo*.[20,22] Phospholipid surface modification can also decrease tendon gliding resistance and improve outcome after flexor tendon repair.[18] Recently, the addition of lubricin has demonstrated dramatic improvement in extrasynovial tendon gliding ability in a canine *in vitro* model.[19] All 3 lubricating molecules exist in native synovial fluid (SF) and are found on the intrasynovial tendon surface; each has an important role in joint and tendon lubrication.[23–30]

In this study, we used native SF as a lubricating material and used a relatively simple tissue engineering technique, carbodiimide derivatization, to fix the SF onto an extrasynovial tendon surface in a canine model *in vitro*, to test the hypothesis that fixation of SF decreases gliding resistance and smooths the tendon surface.

MATERIALS AND METHODS

Specimen preparation

We harvested 63 peroneus longus (PL) tendons from the hind legs of 32 adult mongrel dogs, weighing approximately 20 kg each, which were killed for other studies approved by our Institutional Animal Care and Use Committee. The superficial aspect of the paratenon around the PL tendon was carefully removed, as recommended clinically when extrasynovial tendons are used for tendon grafting.[16] The second digit was dissected from each hind paw. The proximal pulley, proximal and middle phalanx, and flexor digitorum superficialis tendon insertion was carefully preserved and the flexor digitorum profundus tendon was removed. Then the proximal interphalangeal joint was fixed in full extension with a longitudinal 2-mm K-wire (Fig. 1). We immediately assessed 3 PL tendons for morphological evaluation using scanning electron microscopy (SEM); these tendons served as the normal tendon group. We randomly assigned the other 60 PL tendons to 6 groups of 10 tendons each. Each group was then treated by one of the 6 solutions described below and then evaluated mechanically and morphologically.

Control (saline)—We treated the tendons with 0.9% NaCl, 0.1 mol/L Mes (Sigma Chemical, St. Louis, MO), pH 6.0.

Carbodiimide derivatization only (cd only)—We treated the tendons with 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Sigma Chemical)[31]

and 1% *N*-hydroxysuccinimide (NHS; Pierce, Rockford, IL), 0.1 mol/L Mes, pH 6.0. In the carbodiimide derivatization reaction, EDC couples carboxylic acid groups to primary animo groups, resulting in the formation of amide bond (Fig. 2). This crosslinking reaction is usually performed between pH 4.5 and 7.5 and requires only a few minutes for completion.[32]

Carbodiimide derivatization plus gelatin (cd-G)—We treated the tendons with 10% gelatin (Sigma Chemical), 1% EDC, 1% NHS, 0.1 mol/L Mes, pH 6.0.

Carbodiimide derivatized synovial fluid plus gelatin (cd-SF-G)—We treated the tendons with 88% SF, 10% gelatin, 1% EDC, 1% NHS, 0.1 mol/L Mes, pH 6.0.

SF Only—We treated the tendons with 100% SF, 0.1 mol/L Mes, pH 6.0.

Carbodiimide derivatized SF (cd-SF)—We treated the tendons with 98% SF, 1% EDC, 1% NHS, 0.1 mol/L Mes, pH 6.0.

Treatment of tendon surface

We collected 1 to 2 mL SF from knee joints immediately after the dogs were killed and stored it at −80°C for future use. After we collected a total of 60 mL SF, we defrosted and pooled it and divided it into 2-mL aliquots, each stored in a separate vial and frozen again. Just before each experiment, a synovial bottle was randomly chosen, defrosted, and then used for tendon surface modification. The PL tendons were immersed for 30 seconds in one of the 6 solutions. After immersion, each tendon was placed in an incubator at 100% humidity at 37°C for 15 minutes, wrapped with wet towels, kept at 37°C for 1 hour, and then tested.

Measurement of friction force

We evaluated the gliding resistance between the PL tendon and proximal pulley using a testing device developed previously.[33–35] A normal canine proximal phalanx with the intact A2 pulley was secured on the custom-made device with the volar side upward (Fig. 1) in a saline bath (ISOTEMP 202; Fisher Scientific, Houston, TX) at 37°C. Each tendon was tested against its own phalanx and pulley. The tendon was then pulled at a rate of 2 mm/s by the actuator against the weight with a fixed excursion of 16 mm, an estimate of normal canine tendon excursion distance based on previous studies.[36] The experimental setup consists of one mechanical actuator, a linear potentiometer, and a tensile load transducer, which were connected to the proximal tendon end. The resolution of the recorded output of the force transducers in this setup is less than 1 g. Another tensile load transducer, a pulley unit, and a 4.9-N weight were connected to the distal tendon end. The motion between tendon and pulley was repeated for 1,000 cycles. The number of simulated repetitive cycles, 1,000, was determined based on the estimated total number of postoperative passive motions in a 6-week *in vivo* canine tendon rehabilitation model.[22,36,37] The data of the gliding resistance were recorded after every 50 cycles up to 500 cycles, and then every 100 cycles up to 1,000 cycles. The first 2 cycles of frictional testing were used as preconditioning and to remove any loose superficial reagent; we collected data from the third cycle motion as the first data collection cycle. The force difference between the proximal and distal tendon ends represents the gliding resistance of the flexor digitorum profundus tendon against the A2 pulley. The gliding resistance was obtained from the equation $(F2_{flexion} - F2_{extension})/2$.[38]

Evaluating by SEM

After 1,000 cycles of testing, we prepared 3 tendons in each group for SEM. The selected tendons were washed in phosphate-buffered saline solution and fixed in a solution of buffered glutaraldehyde and osmium tetroxide. After dehydration in graded acetone, the specimens were coated with gold/palladium alloy and examined by SEM. The specimens were mounted on a Hitachi 4700 scanning electron microscope at 3 kV. The surface of the tendon was qualitatively assessed, paying particular attention to the smoothness of the tendon. As noted above, 3 PL tendons were evaluated as a normal extrasynovial tendon just after death.

Statistical analysis

We determined the mean ± SD of the gliding resistance for each treatment group. We used one-way analysis of variance to compare friction data among the 6 experimental groups. For effects of interest, least square means, differences in means, and 95% confidence intervals were determined. We used Tukey-Kramer post-hoc test for each pairwise comparison when there was a significant difference. A p value less than .05 was considered to indicate statistical significance in all cases.

RESULTS

There was no significant difference in gliding resistance of the PL tendon before treatment among the 6 experimental groups (Fig. 3). After 1,000 cycles of tendon gliding motion, the gliding resistance in saline, cd only, cd-G, cd-SF-G, SF only, and cd-SF groups was 0.92 ± 0.02, 0.93 ± 0.06, 0.50 ± 0.11, 0.20 ± 0.06, 0.84 ± 0.12, and 0.79 ± 0.13 N, respectively (Fig. 3). We found a significant difference in gliding resistance between the first cycle and 1,000 cycles in all groups ($p<.05$) except the cd-SF-G group (Fig. 3). There was no significant difference in gliding resistance after 1,000 cycles between the saline control group and those with tendon treated with cd only and SF only, but gliding resistance in the cd-G, cd-SF-G, and cd-SF groups was significant lower than in the saline control group after 1,000 cycles of tendon motion ($p<.05$) (Fig. 3). In addition, gliding resistance in the cd-SF-G group was significantly lower than in the cd-G and cd-SF groups ($p<.05$). Tendons treated with cd-SF-G showed the lowest gliding resistance after 1,000 cycles of tendon motion, increasing by 25% compared with the first cycle; whereas that of the saline control group increased 608%.

Figure 4 shows the trend of gliding resistance for each group. Gliding resistance of the PL tendons treated with saline, cd only, SF only, and cd-SF groups increased more over the first 500 cycles than in the next 500. Gliding resistance of the PL tendon in the cd-G group increased at a more gradual rate over the 1,000 cycles. Gliding resistance of the PL tendon treated with cd-SF-G was the most stable over the 1,000 cycles.

On SEM evaluation at low magnification (Fig. 5), the tendon surface after 1,000 cycles of motion in the saline control, cd only, SF only, and cd-SF groups appeared to be rough, with irregularly arranged collagen bundles around the tendon surface, whereas that of the tendons treated with cd-G and cd-SF-G were still smooth. At high SEM magnification, the collagen was fully exposed on the surface of normal PL, saline control, cd only, SF only, and cd-SF groups after 1,000 cycles of tendon motion, but in the cd-G and cd-SF-G groups the collagen was not exposed (Fig. 6).

DISCUSSION

Because of its smooth surface, low friction, and durability, an intrasynovial tendon is the ideal graft tendon for reconstruction of finger function.[14,37,39] The 3 principal biological

lubricants are hyaluronic acid, lubricin, and phospholipids.[23,30,40] Lubricin is the principal lubricant in joints.[41] Phospholipids not only function as a joint lubricant but also lubricate other gliding surfaces[42,43] including tendon.[29,30] Improvement in tendon gliding ability by applying each of these biological lubricants has been reported. Momose et al[44] reported that surface modification of extrasynovial tendon with carbodiimide derivatized hyaluronic acid improved tendon gliding ability, and that SF simply placed on the PL tendon surface did not bind strongly enough to the PL tendon surface, so that it was easily rubbed away with repeated tendon motion. Other studies have confirmed these results in extrasynovial and intrasynovial tendon and after tendon repair and graft, both *in vitro* and *in vivo.*[15,19–22,45,46] Recently Taguchi et al[19] combined 2 lubricating molecules, hyaluronic acid and lubricin, and found better results compared with either lubricant used alone.

Our results demonstrated that surface modification using cd-SF-G significantly improved extrasynovial tendon gliding ability during 1,000 cycles of tendon motion. In addition to the natural combination of biological lubricants in SF, the other advantages of using native SF are that it is easily obtained and cost-effective. The same principle could be further investigated in other applications, such as treating arthritic joints. This study also emphasizes the role of gelatin in improving gliding resistance over and above the effect of the lubricant, especially when looking at the effect over 1,000 cycles. We believe that this effect is a result of gelatin working as a grout, filling the interstices of the surface collagen network. In addition, the gelatin may provide more binding sites for the carbodiimide-activated lubricant.

There are some limitations to this study. First, it is an *in vitro* investigation, which may not apply to an *in vivo* study. The biological effect of this intervention on tendon graft function *in vivo* is not known. Second, although we used SEM to identify smoothness on the tendon surface, we did not perform a quantitative evaluation. Third, other tendon mechanical properties such as tensile or compressive moduli were not assessed at this time. Finally, other than surface imaging, we performed no surface assay to confirm that crosslinked SF remained on the tendon surface.

Surface modification of extrasynovial tendons with carbodiimide derivatized SF plus gelatin improves tendon gliding ability *in vitro*. If confirmed *in vivo*, this finding may have implications for clinical tendon graft surgery.

Acknowledgments

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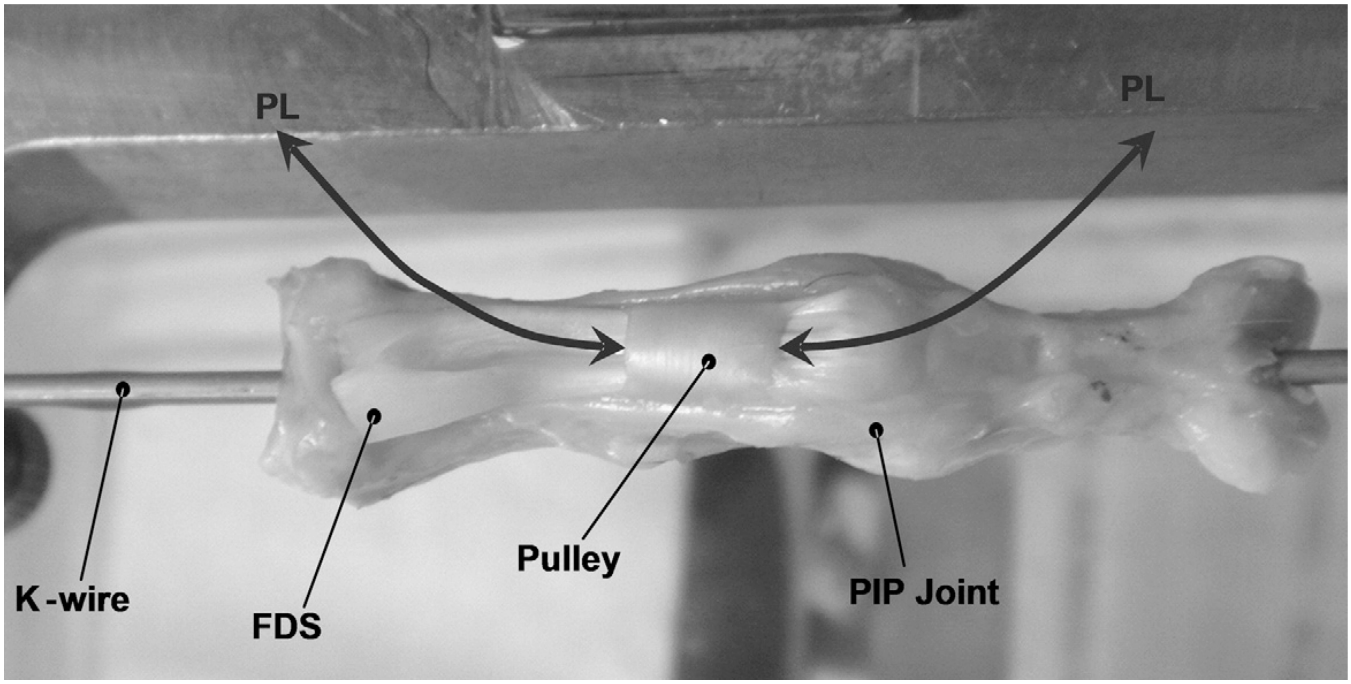

FIGURE 1.
Pulley unit: The proximal phalanx and intact A2 pulley were mounted on the device and fixed in full extension of the proximal interphalangeal joint by a longitudinal K-wire.

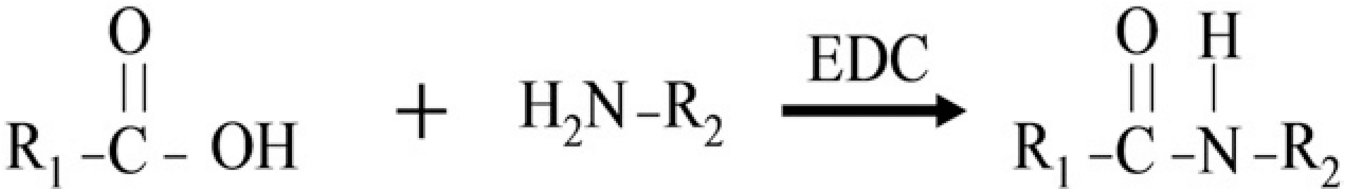

FIGURE 2.
Carbodiimide derivitization reaction.

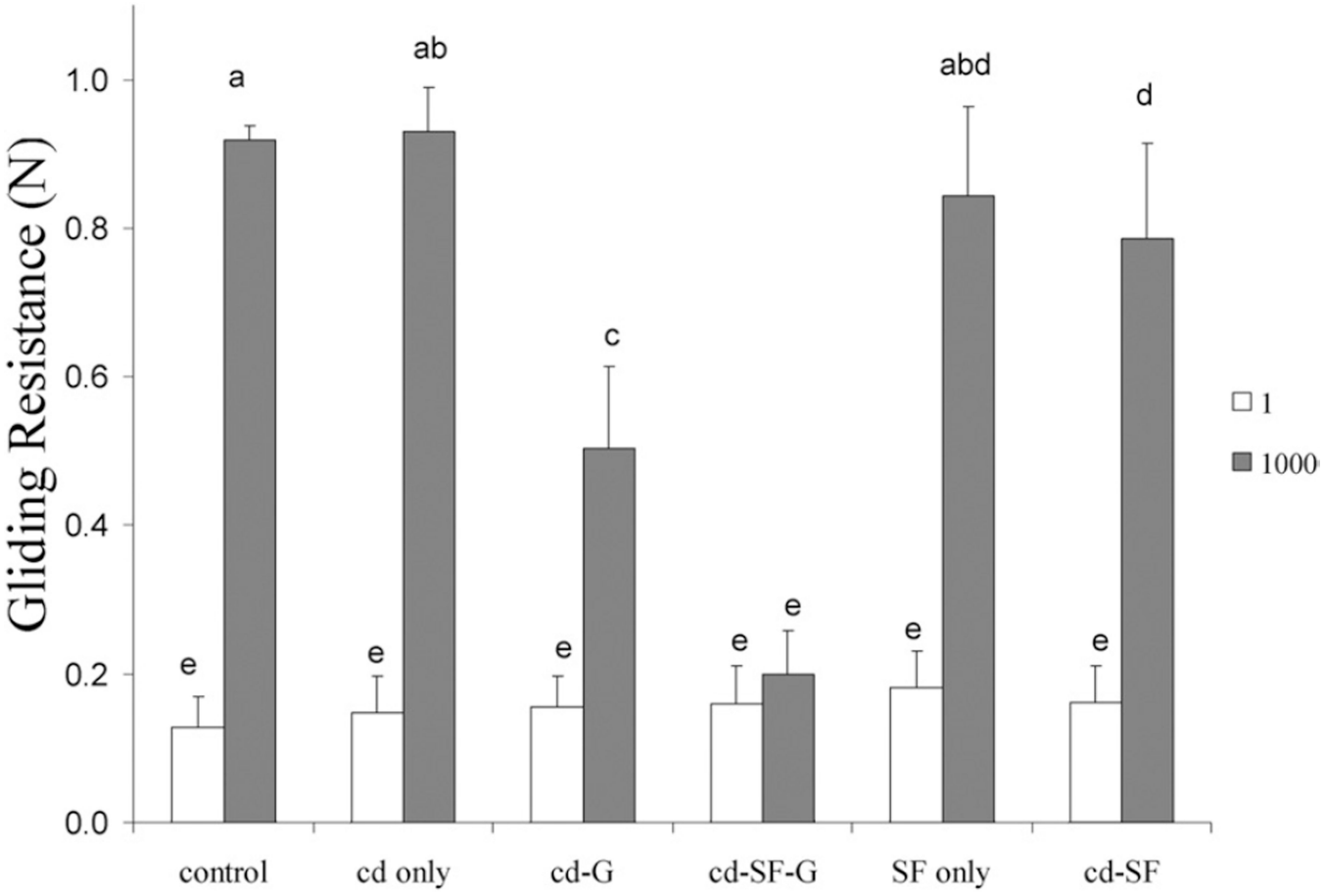

FIGURE 3.
Frictional force at first cycle and 1,000 cycles in each group. Different letters indicate significant differences ($p<.05$).

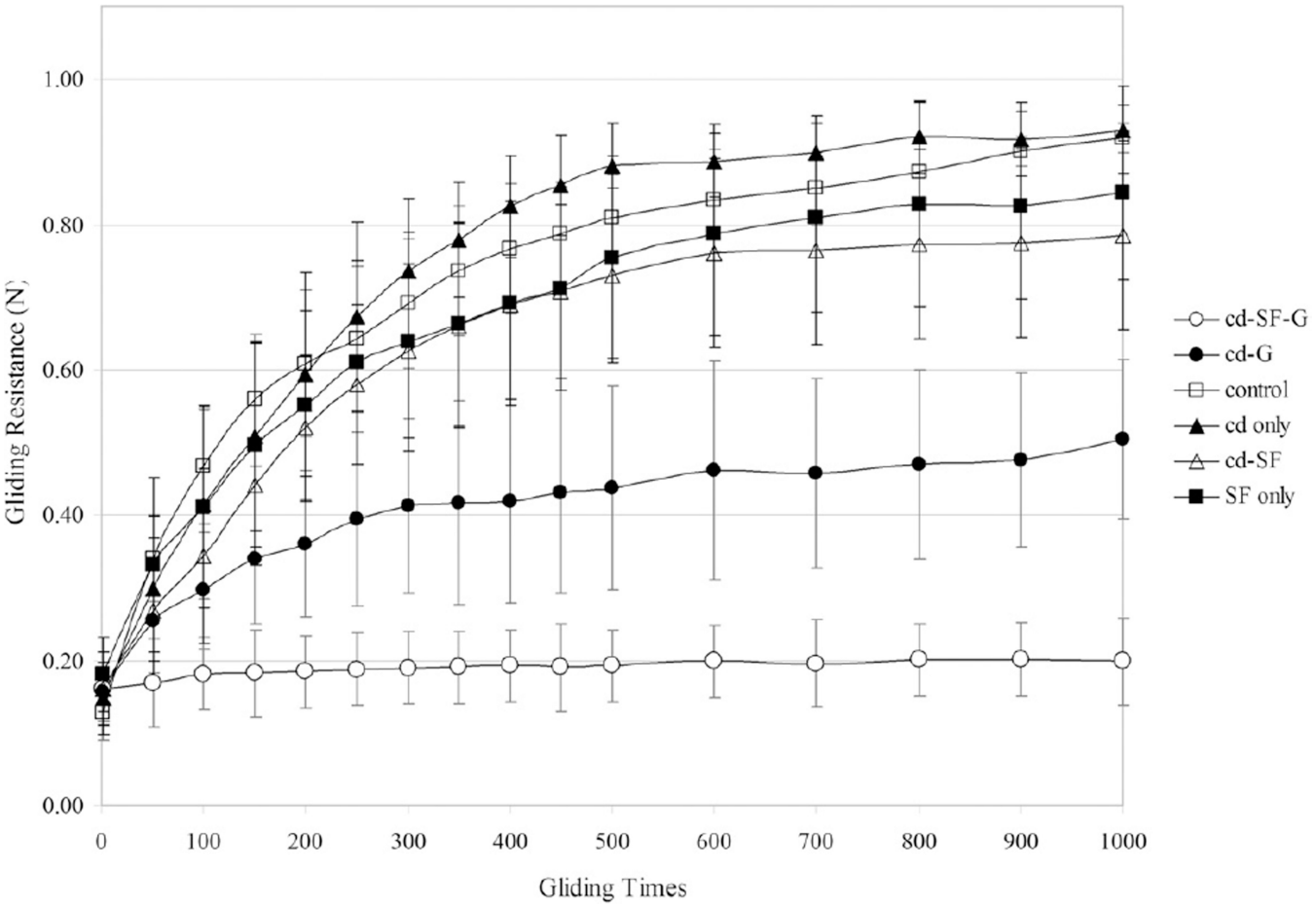

FIGURE 4.
The trend of gliding resistance from the first cycle to 1,000 cycles.

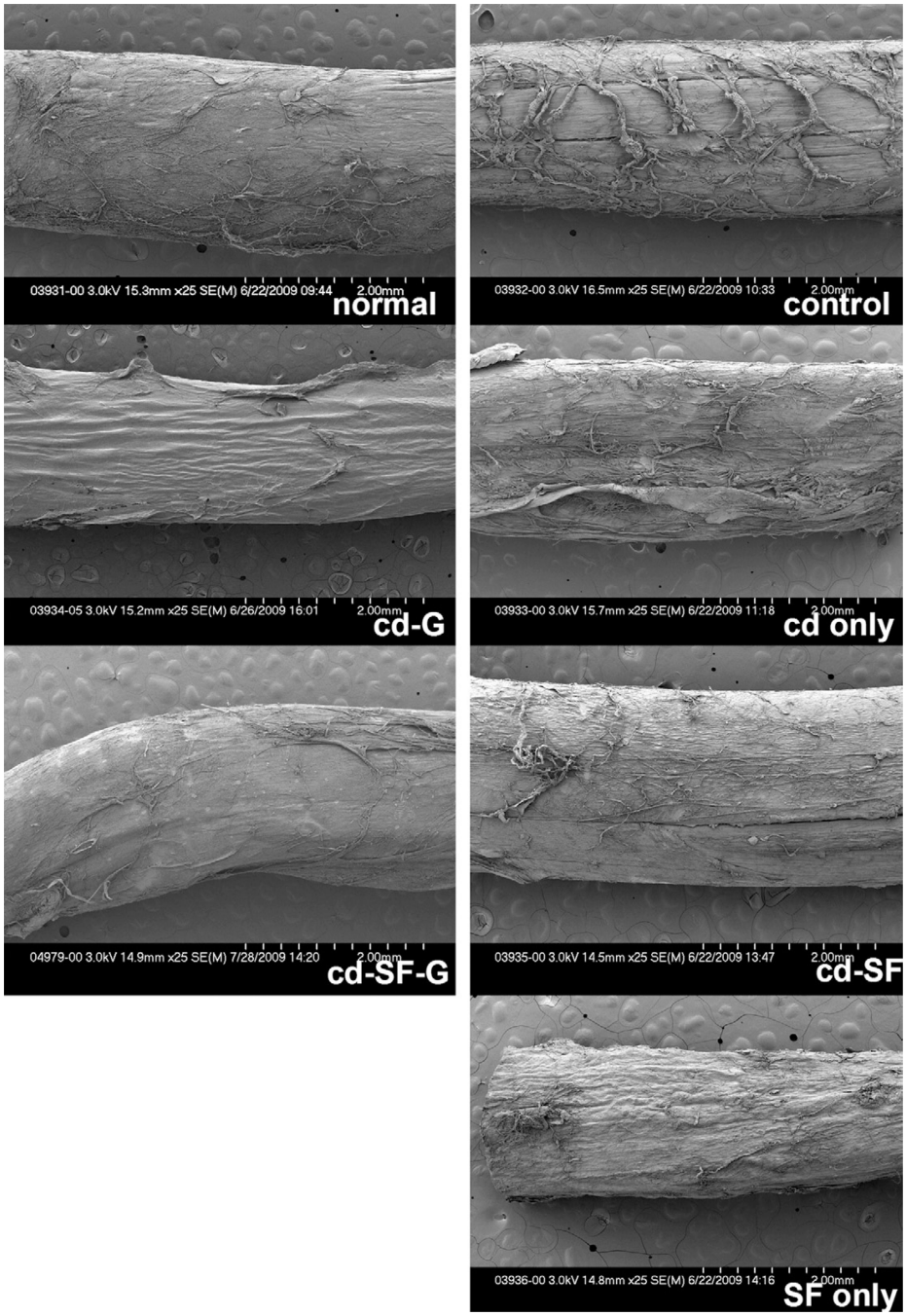

FIGURE 5.

Scanning electron microscope images after 1,000 cycles of motion. (Low magnification, ×25.) A smoother surface was noted in the groups treated with cd-SF-gelatin and cd-gelatin.

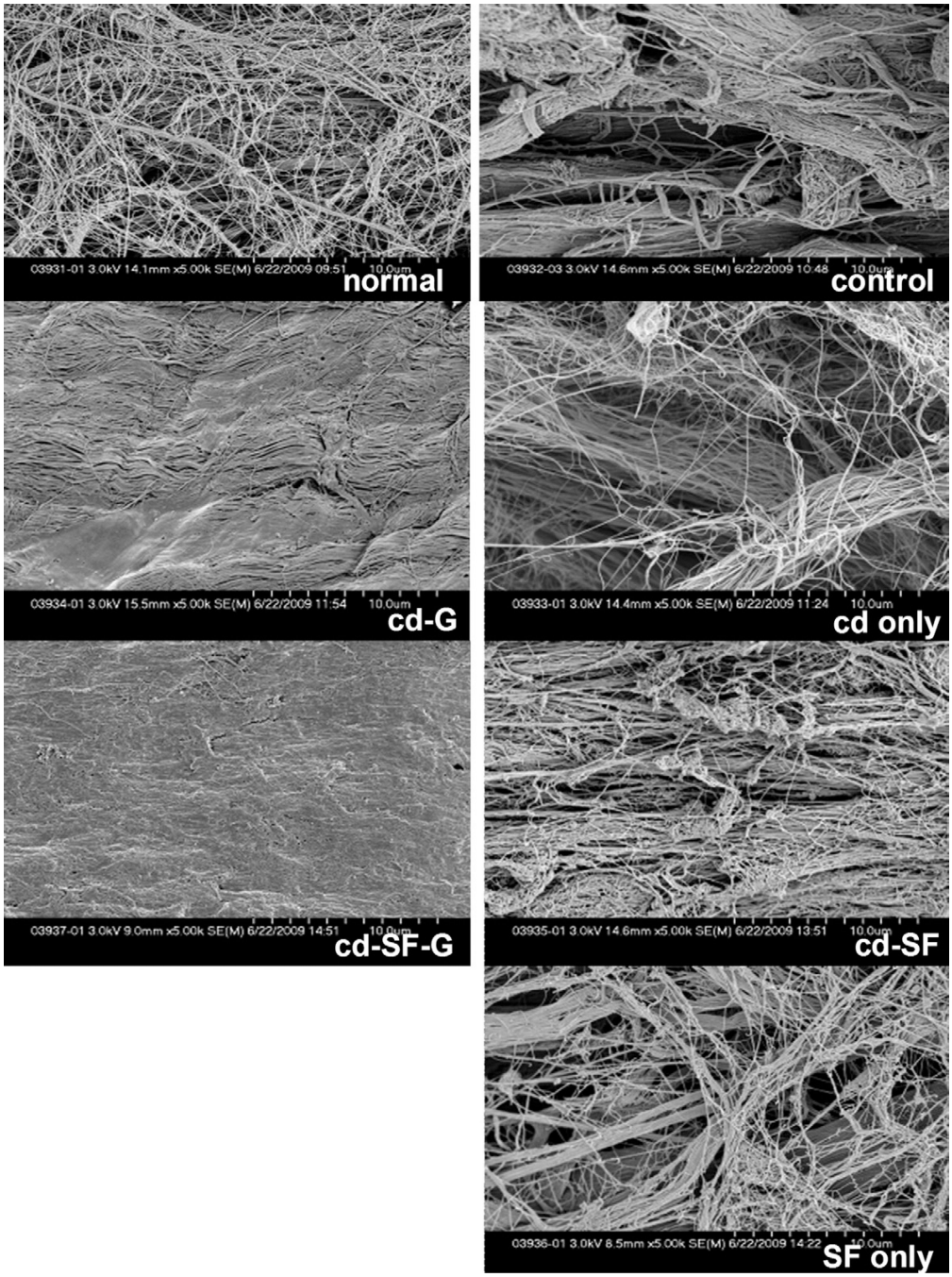

FIGURE 6.

Scanning electron microscope images after 1,000 cycles of motion. (High magnification, ×5,000.) A smoother surface was noted in the groups treated with cd-SF-gelatin and cd-gelatin.