Introduction: Flexor tendon repair has been a challenging clinical problem. Failure to achieve primary healing leads to gapping and rupture which results in scar adhesions, restriction of gliding, and significant disability. Much research has focused on tendon to tendon healing which showed decreased tensile strength of the repair site 4-5 days post surgery and improved tensile strength at 3 weeks (1-3) and via histologic observations (4). More recent research has focused on healing mechanisms at the molecular level including the role of collagenolytic enzymes (5,6). The risk of failure in tendon repair is high because no suture method preserves its tensile strength throughout the time required for tendon ends to heal strongly to each other while allowing the repairing tendon to glide. In spite of the fact that failure is most often due to suture pullout, no significant research has been focused on the site where sutures interface with tendon tissue. The purpose of this study was to: (1) determine and compare the tensile strength of the suture/tendon interface at various times post surgery, (2) characterize the histologic changes at the suture/tendon interface, and (3) develop an in vitro enzymatic assay to test for enzymatic activity at the suture/tendon site.

Methods: Forty chickens were included in the study. A 4.0 Mersilene suture was placed in the deep flexor tendon of each toe. The Savage and Kessler suture patterns were used. In order to examine only data from suture/tendon interface sites, the tendons were not cut. Thus, there was no healing response to confound the results. Animals were sacrificed on postoperative days (POD) 0, 1, 3, 6, 10, 14, 21 and 28. Toes were dissected, and tendon cross sectional area measured. Tensile testing was performed on a 1321 Instron Materials Testing equipment at a displacement rate of 2.5 cm/min. The peak load and elongation at the time of suture pullout from the tendon was recorded. Selected specimens were fixed in formalin and stained with H&E and Masson’s trichrome. Statistical analysis was performed using a weighted 2-way ANOVA and regression analysis for POD 0-6 and POD 0-28.

Tendon samples containing the suture site were frozen in liquid nitrogen and pulverized in a Spex freezer mill. Pulverized tissues were extracted in a Triton x-100 buffer and then in a high calcium buffer. After clarifying by centrifugation at 20,000 x G, the extract was concentrated using a Microcon filtration centrifugation tube system. Protein concentration was measured using the BioRad Protein Assay.

Protein samples were then incubated with 14C-labeled Type I or Type III collagen for 48 hours using APMA as an activator at 32°C. Products were fractionated by SDS-PAGE at 150V, and the gels fixed, dried and exposed to X-ray film at –80°C for 48 hours. In addition, gelatin zymography was performed. Protein extract samples were electrophoresed on gels containing 0.1% gelatin. The gels were incubated in Triton X-100 buffer to remove the SDS, incubated overnight at 37°C in Collagenase buffer, and then stained with 0.006% Coomassie Blue.

Results: Evaluation of the biomechanical data for the Savage pattern revealed a decrease in tensile strength over POD 1 through POD 6 compared to POD 0. An increase in strength compared to POD 6 was noted at POD 28. The ANOVA showed differences in the mean peak load/area during POD 0-6 (p<0.06) Regression analysis using a linear model demonstrated a downward trend in tensile strength over POD 0-6 (p<0.01) (Fig. 1). A quadratic model also revealed a decrease over POD 0-6 with an improvement in strength at POD 28 (p<0.02) (Fig. 2). Similar evaluation of the Kessler pattern data showed no definite trends in the tensile strength over time.

Histologically, tendons with the Savage pattern showed no changes for POD 0 and appeared as normal tendon tissue. POD 3 and 6 were characterized by a noticeable decrease in cells around the suture site. Disruption of the adjacent collagen fibers was seen. By POD 10, there was a marked increase in cellular activity at the suture site. The cells were primarily fibroblasts and inflammatory cells. The increased number of fibroblasts persisted throughout POD 21 and 28.

Using the in vitro assay, the presence of both collagenase and gelatinase was detectable. Both Type I and Type III collagens were lysed by a collagenase. The presence of typical collagenolytic products (TCs) was especially noticeable at POD 1 and POD 21. Lesser degrees of activity were seen at POD 3-10 (Fig. 3). The gelatinase assay revealed the presence of a 72 kDa gelatinase. Levels of this gelatinase were detectable on POD 6-28. No activity was noted on POD 0-3 (Fig. 4). Similar profiles of enzyme activity were seen with the Kessler pattern.

Discussion: This study evaluated interactions at the suture/tendon interface. The clear difference in tensile strength over the first 6 days post op is similar to that previously described for the Savage technique. Technical considerations may account for lack of differences noted with the Kessler pattern. The histologic changes appear to show a delay in cell migration compared with prior observations at the tendon/tendon healing site. The presence of enzyme activity to both Type I and Type III collagen suggests collagenase from more than one cell type or an enzyme capable of digesting both types of collagen. The 72 kDa gelatinase is characteristic of Gelatinase A which is produced primarily by fibroblasts. A better understanding of the biologic events that take place at the suture/tendon interface will improve the outcomes in tendon repair surgery; therefore, additional research is warranted.

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