A NOVEL MURINE MODEL OF FLEXOR TENDON GRAFTING AND ADHESION FORMATION

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Tendon repair is complicated by fibrotic adhesions that compromise the tendon gliding function. Here we seek to elucidate the differences in adhesion formation in autograft vs. allograft repair of the murine FDL tendon.

METHODS: The distal FDL tendon was transected; a freeze-dried tendon allograft or a live autograft was used to repair the gap. Mice were sacrificed, at multiple end points, up to 84 days post surgery. The limb was fixed and the FDL tendon was incrementally loaded. The MTP flexion angle was quantified at every load. The flexion angle was plotted vs. the excursion load. The rate constant of the rise of the curve (α), represents the resistance to flexion and is therefore termed *the adhesion coefficient*. Following, the tendon was tested for biomechanical properties.

RESULTS: The adhesion coefficient at 14 days was greater than normal for both auto- and allograft ($p<0.001$). No significant difference, between the grafts, was seen at 14 days. At 28 days, the adhesion coefficient of the autografts was 40-folds greater than normal tendon ($p<0.001$), and for allografts the adhesion coefficient was increased 20-fold compared to normal tendon ($p<0.001$). By 42 days and thereafter, the adhesion coefficients decreased significantly. Surprisingly, there were no significant differences in maximum tensile force or stiffness between auto- and allograft repairs. While there were mild improvements over time, the tensile strength never exceeded 50% of normal.

CONCLUSION: This model offers a tool to examine the biomechanical features, as well as cellular and molecular events, associated with tendon repair and adhesion formation. Further, it suggests that allografts may offer a clinically favorable alternative due to the lack of difference in biomechanical properties and adhesion formation.

INTRODUCTION:

Tendon, ligament, and joint capsular injuries represent 45% of the nearly 33 million musculoskeletal injuries occurring each year in the United States, and hand injuries account for 5-10% of annual emergency department visits nation wide (Praemer, 1999). Common among these injuries are flexor tendon lacerations and ruptures, especially in individuals active in sport.

Repair of injuries to flexor tendons of the hand is complicated by fibrotic adhesions that compromise the tendon gliding function (Taras, 1999). To date, restoring the gliding function after primary repair of flexor tendon injuries, especially in Bunnell's "no man's land" or zone II (Bunnell, 1953), remains an unsolved problem (Chang, 2000). In zone II flexor digitorum profundus (FDL) passes through the tail of the flexor digitorum superficialis and the two tendons glide in a tough fibrous sheath or retinaculum. As an alternative to primary repair, surgeons often use a tendon autograft, passing it through the flexor sheath tunnel of zone II, and surgically attaching the proximal and distal graft ends outside of zone II. Indications for flexor tendon grafting include cases where flexor tendon repair has been delayed because of infection. Other reasons for tendon grafting are cases when tendons have ruptured and the severed ends are nonviable, such that they can't be effectively sutured together, or cases when a primary repair has failed. At such indications, continuity and gliding function can often be restored by using a graft to bridge the defect (Stark, 1989). The use of autograft and allograft tendons in single or two-staged flexor system reconstruction is increasing especially as a late management option for neglected flexor tendon injuries (Beris, 2003; Coyle, 2002; (Leversedge, 2000; Liu 1997; Sakellarides, 1996; Tolat, 1993).

However, flexor tendon grafting procedures are not without pitfalls, and they can be very challenging even for the most experienced hand surgeon. The most persistent problem with tendon grafting is the adhesion formation. Adhesions occur whenever the surface of the live tendon graft has been violated, either through intrinsic fibrosis (suturing or surgical manipulation) or through extrinsic fibrosis (whenever the tendon sheath is disrupted). The extent to which adhesions arise from intrinsic or extrinsic factors remains unclear. Despite being studied for decades in various animal models, the biological mechanisms of flexor tendon grafting and adhesion formation are still poorly understood. Some believe that flexor tendons have an intrinsic capability for healing (Singer, 1989; Wu, 2000; Seiler, 1997), while others disagree and believe that healing is extrinsically mediated by the fibroblastic and

mesenchymal cells of the tendon paratenon and surrounding synovial sheath (Potenza,1982).

Autograft tendon reconstruction may exacerbate adhesion formation, due to proliferation of autograft tenocytes and migration of cells away from the tendon, along with other intrinsic and extrinsic factors (Taras, 1999). Based on these observations, we hypothesize that nonviable allograft can be manipulated without inducing the insult that precedes the fibrosis and adhesions in live autografts. Few reports have investigated adhesion in flexor tendon allografts, some seem to support the premise of this hypothesis. Ramesh et al. compared bovine superficial digital flexor tendon acellular allografts, fresh autografts, and glutaraldehyde-preserved allografts and reported that early organization, minimal adhesion formation and lesser thickening of tendon at the reconstructive site in the acellular allograft (Ramesh, 2003).

In this study the hypothesis that an acellular tendon allograft heals with less fibrotic adhesions than a live autograft was tested. A novel murine FDL tendon grafting model was created to quantify adhesion formation and measure biomechanical properties. A gap in the flexor digitorum longus (FDL) was reconstructed with either an auto- or allograft. The data shows the potential for freeze-dried allografts to reduce adhesion formation and to improve the outcomes of tendon repair.

METHODS:

Animal Surgeries: Studies were approved by the University Committee for Animal Resources. Eight-week old C57BL/6 mice where randomized into two experimental groups; allografts and autografts. The mice where anestetheized with ketamin (60mg/kg)/xyaline (4mg/kg) via a intra-peritoneal injection. Surgeries were preformed using aseptic technique. A longitudinal plantar incision was made on the left hind foot. The distal FDL tendon of the mouse was isolated and transected on the plantar surface of the metatarsal. A 3 mm freeze-dried tendon allograft, or freshly harvested live autograft from the contralateral foot, was sutured between the ends of host tendon using modified Kessler technique and a 8-0 nylon suture. The tendon was then transected at the proximal musculotendinous junction to temporarily immobilize the flexor mechanism, to prevent disruption of the tendon graft early during the repair period, and to induce adhesion formation. The skin was closed with 4-0 silk suture. Buprenorphine was used for post-operative analgesia. Mice were sacrificed at 0, 14, 21, 28, 42, 63 and 84 days post surgery for adhesion testing and biomechanical evaluation. There were 9-12 animals in each group.

Freeze-drying procedure: The FDL allografts were harvested from donor mice using aseptic technique, rinsed in ethanol, snap frozen in liquid nitrogen and placed in the freeze-drying chamber. The tendons were freeze-dried according to the manufacturer's specifications for 12 hours. After freeze-drying, the tendons were stored at -80C until the day of surgery, as previously done (Koefoed, 2005; ito, 2005). Before grafting, the allografts were reconstituted with sterile normal saline.

Adhesion Test: To evaluate the metatarsophalangeal (MTP) joint flexion range of motion (ROM) and the resistance to flexion due to adhesion formation, an adhesion test was preformed. The lower hind limb was disarticulated from the knee, the limb was frozen in normal sterile saline and kept at -85 C. 1-7 days later the limbs where thawed for testing. An incision was made on the medial side. The FDL tendon was isolated and transected at the musculotendinous junction, and dissected free to just proximally of the tarsal tunnel without disrupting the skin at the ankle or foot. The proximal end of the tendon was sandwiched between two pieces of tape using super glue. A hook and a line were passed through the tendon-tape sandwich. The lower hind limb was fixed in a custom holding apparatus, where the tibia was rigidly held. The plantar surface of the foot was rested against a flat surface to prevent plantar flexion. The FDL tendon was incrementally loaded using dead weights (0 – 19 grams). The toes were allowed to flex to an equilibrium position at every incremental load application and digital images were taken medially at each applied load to quantify the MTP flexion angle relative to the zero-load neutral position (Figure 1-a).

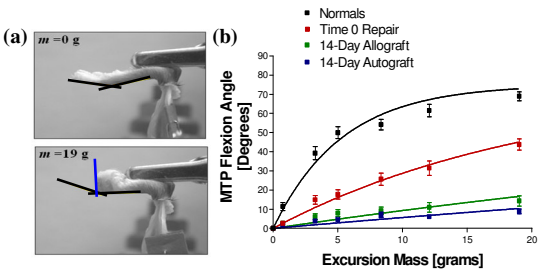

Figure 1. Adhesion testing of grafted FDL tendons in the mouse model.

The digital images were subsequently transferred to a computer and the MTP joint flexion angle and ROM (the ROM was measured as the maximal MTP joint flexion angle) were measured with ImageJ software (http://rsp.info.nih.gov/ij). This was done in a blinded fashion by two observers making two measurements each. The MTP joint flexion angle was plotted versus the applied excursion loads (Figure 1-b). Based on the flexion curve of the normal tendon, the flexion data were fitted to a single-phase exponential association equation:

$$\text{MTP Flexion Angle} = \beta \times [1 - \exp(-m/\alpha)]$$

where m is the applied excursion load. The curve fit was physically constrained by a maximum flexion angle (β) that was set to the maximum flexion angle for normal tendons (75°). The rate constant of the rise of the flexion curve (α) is representative of the resistance to flexion due to adhesions, and is therefore termed the adhesion coefficient. This novel adhesion coefficient represents the first tool for investigating flexor tendon scarring and adhesions.

Biomechanical Testing: Following the adhesion test, the FDL tendon was released from the tarsal tunnel, and the foot severed at the calcaneus, leaving the tendon attached to the phalanges and metatarsals. The FDL tendon was dissected free from remaining soft tissue and placed in sterile gauze soaked with saline to maintain adequate tissue hydration. The FDL tendon was then mounted on the Instron 8841 DynaMight™ axial servohydraulic testing system (Instron Corporation, Norwood, MA) using custom grips. The tendon was loaded in tension in displacement control at a rate of 30 mm/minute until failure. Force-displacement data were plotted and the biomechanical properties, including the maximum tensile force and stiffness, were determined.

RESULTS:

The adhesion coefficient for day 0, meaning no healing allowed, was increased 5-fold compared to normal tendon (n=8). That applies to both auto- and allografts (n=9 and n=9) ($p>0.05$). The adhesion coefficient 14 days post grafting was 29- and 26-folds greater than normal FDL tendon for auto- and allografts, respectively (n=12 and n=12) ($p<0.001$). 21 days post grafting, the adhesion coefficient drops to 9- and 8-folds normal FDL tendon auto and allograft respectively (n=23 and n=22) ($p<0.05$). At 28 days post grafting, the adhesion coefficient rises to 40- and 20 folds normal FDL tendon, auto- and allograft respectively (n=22 and n=23) ($p<0.001$). By 42 days and thereafter, the adhesion coefficients significantly decreased in both auto- and allografts (n= 10-12) but remained higher than normal FDL tendon. Remarkably, no significant difference between auto- and allograft tendon adhesions was seen at any time point (Figure 2).

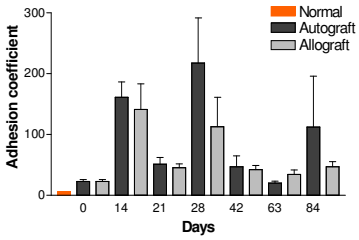

Figure 2. Adhesion Coefficient

A few grafts where excluded from the analysis, altogether 8 autografts and 6 allografts where excluded. One tendon was excluded due to a mistake while testing, two did not show any flexion and a failure in the process was assumed. The exclusion of the remaining tendons was due to a small inversion of the foot making it impossible to measure the flexion angel. The 21 and 28 day group consists of data from two pooled groups and the number of animals was therefore higher.

Surprisingly, no significant difference in maximum tensile force or stiffness between fresh autograft and freeze-dried allograft repairs was seen at any time point up to 84 days post-surgery. While there were mild improvements over time in the tensile strength (as indicated by the maximum tensile force at failure), both auto- and allograft repairs never exceeded 50% of normal strength (figure 3). The stiffness for both auto- and allograft repairs, increased significantly over time, reaching 75-90% of the stiffness of normal non-grafted FDL tendon. Each group consisted of 9-12 animals, only 2 animals where excluded; 1 autograft and 1 allograft.

The tendons failed at different sites, most failed in the area of the proximal or distal suture, a few failed mid-graft, and only one tendon, a 42 day allograft, failed at the grip. We saw no difference in failure site among the groups.

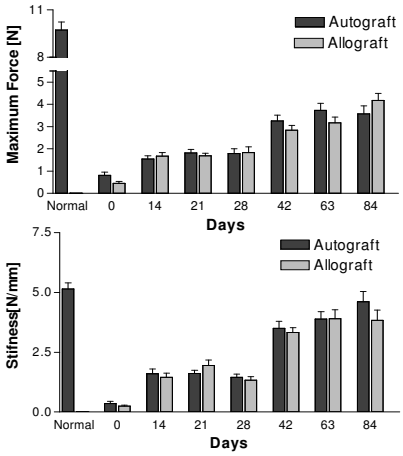

Figure 3. Biomechanical properties.

DISCUSSION:

The current study demonstrates that reconstruction of tendon defects with either autograft or allograft tendon results in the formation of significant adhesions. When quantifying the adhesions, no difference between autograft and allograft adhesion formation was seen, indicating that intrinsic healing play a little, if any, role in tendon grafting. A number of factors could have influenced this result. First, even though the autograft was kept moist, it could have dried out during the operation, this would damage the tenocytes. Second, our method could lack accuracy in detecting a possible difference. Third, the autograft surgeries where always performed first, in other words the surgeon could be less focused at the allograft surgeries, even though this was not noted.

The data of the adhesion coefficient (figure 2) indicates a two faced healing response. This could be caused by the

graft first healing by scarring creating adhesions, followed by a remodeling face where the adhesions might diminish as part of the remodeling.

We measured the MTP flexion ROM (the MTP flexion angle following the application of an excursion load of 19 grams). Figure 4 shows a strong nonlinear correlation (R^2=0.75) between the empirically determined adhesion coefficient and the MTP flexion ROM. This corroborates the validity of the adhesion coefficient as a quantitative measure for resistance to flexion due to adhesion formation.

The biomechanical test shows a mild improvement over time in both maximum force and tensile stiffness for both autograft and allograft. However, as in the adhesion test we see no difference between auto- and allograft. After 84 days of healing the maximal tensile strength is less then 50 % of normal non-grafted tendon. Therefore, it is quite possible that the graft, either autogenous or allogenous, acts as a temporary scaffold, that may be prone to reabsorption, as it remodels to re-establish tendon continuity and restore its role in transmitting loads from muscles to bone to effect joint flexion.

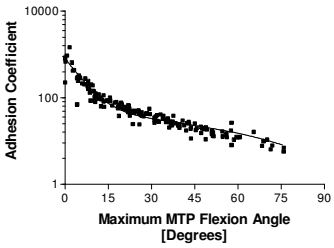

Figure 4. The correlation between ROM and the adhesion coefficient.

Other studies have similar findings. Potenza et al demonstrated that extrinsic cells from the synovial capsule of the joint populated and contribute to the healing of lacerations within freeze-dried allografts implanted in canine and rabbit knee joints (Potenza, 1982). Ramesh et al reported that acellular allografts induce minimal adhesion formation in bovine flexor tendons (Ramesh, 2003).

Failure mode analysis indicates that almost all the repair specimens failed at either the proximal or distal graft/host junction, which represented the weakest link. Only one of the repair specimens failed at the soft-tissue grips, which confirms that the recorded biomechanical data reflect the average properties of the remodeling repair.

We tested fresh autograft and freeze-dried allograft repairs immediately after transplantation at day 0 (Figure 3). These tests merely measure the pull-out strength of the suture that anchors the graft to the host tendon ends. The average strength and stiffness of the day 0 fresh autograft repair were 0.80 N (± 0.15 SEM), and 0.36 N/mm (± 0.08 SEM), respectively. Similarly, the average strength and stiffness of the Day 0 freeze-dried allograft repair were 0.45 N (± 0.09 SEM), and 0.24 N/mm (± 0.04 SEM), respectively. These values are significantly lower than normal FDL tendon properties, and likely lower than the in vivo forces and excursions the FDL tendon may experience in the mouse model. Therefore, we protected the repair from excessive in vivo loading, which may cause premature failure of the grafts, and hinder healing by a proximal musculotendinous transaction. Transecting the FDL muscle could explain that less than 50% of normal strength was achieved, since the healing response is possibly lowered due to the lack of mechanical stimulation. However, the muscle had healed at the harvest, even after 14 days.

Prior to this study we made a power test and thereby estimated a sample size of 9 animals for the adhesion test and 8 animals for the biomechanical test. To account for additional variability that may arise from differences in activity and post-operative movement, we chose a sample size of 12 animals in each group.

Preliminary data for specimens harvested 28 days post surgery (Figure 5) show that the biomechanical maximal force of fresh autografts or freeze-dried allografts, that were tested for adhesion, were not significantly different from control specimens that were not tested for adhesions (n=5 per group, p>0.05). The stiffness showed similar characteristics (data not shown). From this we could conclude that the non-destructive adhesion test really is non-destructive.

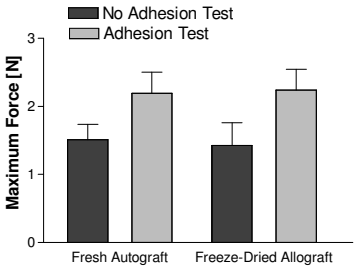

Figure 5. Effect of the adhesion test on biomechanical strength.

Prior to the study we also tested the effects of freeze-drying on the murine FDL tendon. Biomechanical properties of fresh frozen and freeze-dried tendon where tested. Surprisingly, we found mild, albeit statistically insignificant, improvements in maximum force and stiffness for the freeze-dried group (data not included).

CONCLUSION:

The lack of differences in biomechanical behavior between auto- and allografts suggest that processed flexor tendon allografts may offer a clinically favorable alternative to live autografts. In addition to the reduced morbidity associated with the harvest of autograft tendon, human allografting have shown to be more cost efficient than autografting (Cole, 2005). However, this conclusion requires histological conformation, which is in process in our laboratory. Other studies support our findings. Webster et al. compared the healing of flexor tendon autografts and freeze-dried allografts implanted in the paws of dogs. Interestingly, the implanted allografts appeared to be well tolerated by the host and allowed flexor tendon function similar to that allowed by

autografts (Webster, 1983). Others have reported that acellular allografts induce minimal adhesion formation in bovine flexor tendons (Ramesh, 2003).

The present murine FDL tendon model does not represent a true zone II repair model. Nevertheless, it experienced significant adhesions that we quantified and documented using a novel and elegant experimental protocol. Therefore, this novel murine FDL model captures important aspects of the clinical problems associated with flexor tendon adhesions, and can be a powerful tool in elucidating the differences in adhesion formation associated with fresh autografts and acellular, freeze-dried allografts.

Furthermore, the model allows histological testing of the cellular and molecular events involved in repair and subsequent adhesion formation, and can lead to novel therapeutic interventions.

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