Exogenous collagen cross-linking recovers tendon mechanical integrity in an acute tear model.

+ Fessel, G1; Wernli, J1; Li, Y12; Gerber, C1; Snedeker, J G12
+-Orthopedic Research Laboratory, University of Zurich, Balgrist, Switzerland; 1Institute for Biomechanics, ETH Zurich, Switzerland
gfessel@research.balgrist.ch

INTRODUCTION:
 Previous testing of various collagen cross-linking agents showed potential to increase stiffness and failure behavior as well as reduce hysteresis and creep behavior in rat tail tendon fascicles [1]. Based on these results, we hypothesized that exogenous collagen cross-linking might be a candidate for reinforcing injured or healing tendons or to halt progression of tendon tears. The goal of this study was to implement a clinically relevant tendon tear model to investigate tear propagation under repetitive loading as well as to quantify the repercussion of exogenous collagen cross-linking on tear progression.

METHOD:
 Cross-linking effects: Genipin (GEN) and methylglyoxal (MG) were selected based on low toxicity [2]; physiological relevance [3] and their effects on collagen mechanics [1]. Equine superficial flexor digital tendons (SDFT) were dissected with three parallel, equally spaced blades to yield 40x3x1 mm strips. Strips from one strip have similar mechanical properties allowing a blocked or paired analysis. Cross-sectional area of each specimen was assessed using a caliper. One half of each pair was cross-linked using GEN (n=9) or MG (n=15). The other half was designated as a matched (buffer-only) control and both were tested in uniaxial tension until failure. Seven (7) extra pairs were subjected to a creep-test (only GEN). Tendon tear model and cross-linking: Five triplets of SDFT tendon strips were cut with the same device but with 4 blades aligned. One sample from each triplet was randomly allocated to either a control, injury- (tear-) or a cross-linked-injury group. Injury was applied with a biopsy punch (2 mm). Samples were then control incubated for 3 days (control and injury group) or cross-linked with GEN (injury-cross-linked group). Sample surfaces were marked with graphite powder for local strain measurements based on high resolution images that were acquired from a camera mounted with a telecentric lens while fatigue testing on a dynamic tensile testing machine. All triplets were cyclically loaded from 5 MPa to 25 MPa at 10 Hz until failure. Corresponding machine strains were recorded as well as amplitude and number of cycles to failure. Statistical analysis: First, effects of cross-linking on quasi-static ramp to failure properties were assessed by using paired t-tests. Creep rate was analyzed with the Wilcoxon signed-rank test as the data could not be approximated by a normal distribution. Second, the overall effect of injury and cross-linking after injury as assessed by the mean strain during fatigue tests was tested by a two-way randomized ANOVA (blocked for triplets). Third, local strain measurements adjacent to the injury site showed increased local strains due to injury compared to both other groups. Optical strain measurements adjacent to the injury site showed increased local strains at the sides of the lesion and decreased strains above and below, indicating load shunting from injured regions to intact regions. Local strains in the cross-linked-injury group have not yet been analyzed.

RESULTS:
 GEN cross-linked samples showed a trend to higher elastic modulus (16%), significant higher failure stress (26%) and toughness (35%), while having equal failure strains and significant reduced hysteresis (33%) and creep rate (data in tab.1). Further, stress-strain curves of GEN cross-linked samples showed abrupt failure compared to more plastic failure (long plateau) in the native samples (Fig.1, left). MG cross-linking had an opposite effect as elastic modulus (32%) and failure stress (22%) decreased (Tab. 1 and Fig.1, left). Based on these results, further investigations focused on only GEN for potential in arresting tear propagation.

Two-way ANOVA indicated differences in mean tendon strains (p-value < 0.04) and number of cycles to failure (p-value<0.015) during fatigue testing. Post hoc tests revealed increased tissue strains in injured tendons compared to both the control and cross-linked-injury groups (Fig. 1, right). Similarly, the numbers of cycles to failure was reduced due to injury compared to both other groups. Optical strain measurements adjacent to the injury site showed increased local strains at the sides of the lesion and decreased strains above and below, indicating load shunting from injured regions to intact regions. Local strains in the cross-linked-injury group have not yet been analyzed.

REFERENCES: