

Micro- and nanostructure specific X-ray tomography to study collagen regeneration during tendon healing

Introduction: The recovery of the collagen structure following Achilles tendon rupture is poor, resulting in high risk for re-ruptures (1). The loading environment affects the mechanical properties of healing tendons but knowledge regarding how it affects regeneration of the tendon structure is still limited. This study characterizes the effect of reduced in vivo loading on the regenerating 3D micro- and nanoscale tissue structure.

Methods: Rat Achilles tendons were characterized during early healing (1 and 3 weeks) by comparing full cage activity (FL) with immobilization (UL) (N=1/group) (2). The healing tendons were fixed in formalin and measurements conducted in the central part of the callus. The 3D organization of microscale collagen fibers was visualized by phase-contrast microtomography (PC- μ CT) at TOMCAT beamline, PSI (15 keV, 1.63 μ m pixel size), and the 3D organization of nanoscale collagen fibrils by small-angle X-ray scattering tensor tomography (SASTT) (3) at cSAXS beamline, PSI (12.4 keV, 150 μ m beam size).

Results: Unloading during early tendon healing led to generally less collagenous material being formed and a larger presence of adipose tissue (Fig 1.A). The newly formed fibrils and fibers in unloaded tendons were less packed, more disorganized, and less longitudinally oriented along the main axis of the tendon (Fig 1.B).

Discussion: The structural effects due to unloading may explain the reported impaired mechanical competence of the tissue following immobilization during tendon healing (1). Ultimately, this study provides proof of concept of SASTT to study tendon tissue and its potential to investigate other soft collagenous tissues.

(1) Notermans et al., European Cell and Materials, 2021

(2) Hammerman et al., PLoS One, 2018

(3) Liebi et al., Nature, 2015

![Fig 1. A) Microscale organization obtained by PC- μ CT (arrowhead = adipose tissue) and B) nanoscale organization obtained by SASTT (number of glyphs = fibril amount, glyph direction = fibril orientation, glyph colour = degree of fibril orientation) in a 3 weeks unloaded (UL) and fully loaded (FL) healing tendon. 1

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