REDUCED *IN VIVO* LOADING LEADS TO LESS REGENERATION AND AN ALTERED COLLAGEN ORGANIZATION DURING HEALING OF RAT ACHILLES TENDONS

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Introduction

Recovery of the collagen structure after Achilles tendon ruptures are poor, resulting in high risk for re-ruptures [1]. The loading environment has an effect on mechanical properties of healing tendons but there is still limited knowledge regarding how it affects regeneration of the complex tendon structure. 3D organization of collagen fibers can be visualized at the microscale by phase-contrast microtomography (PhC-µCT) and collagen fibrils can be probed at the nanoscale using small-angle X-ray scattering (SAXS). Recently, SAXS tensor tomography (SASTT) was developed, enabling characterization of 3D structure at nanoscale [3]. This study aims to characterize the effect of *in vivo* loading in healing rat Achilles tendons by visualizing the regenerating structure at the micro- and nanoscale in 3D combining SASTT and PhC-µCT.

Methods

Achilles tendons from female Sprague-Dawley rats (10-12 weeks) were transected and allowed to heal while subjected to different *in vivo* loading scenarios [2]; full loading by free cage activity (FL) or unloading by Botox injections combined with steel-orthosis (UL). 3D measurements were performed on healing tendons (1 and 3 weeks, N = 1/group) fixed in formalin. The central part of the healing callus was analyzed by SASTT (cSAXS beamline PSI, 12.4keV, 150µm beam size, 30ms exposure, 6 tilt angles), and the reciprocal space map in each voxel was reconstructed [3] to obtain 3D fibril orientation and structural parameters [4]. PhC-µCT was performed (TOMCAT beamline PSI, 15keV, 4x magnification, 1.63µm pixel size, 33ms exposure) on the same samples and processed with a structure tensor analysis to quantify 3D fiber orientation [5]. 2D SAXS measurements were performed (cSAXS beamline PSI, 12.4keV, 50µm beam size, 30ms exposure) on unfixed, thawed healing tendons (1,2 and 3 weeks, N = 4/group).

Results

Unloading during tendon healing led to generally less material within the callus and a larger percentage of adipose tissue (Fig 1A). It also affected the regenerated fibrils and fibers, by being less packed, more disorganized, and less longitudinally oriented along the main axis of the tendon (Fig 1B). Regenerated fibrils in the unloaded tendon had almost a perpendicular orientation relative to the stumps (Fig 1B). As healing progressed from 1 to 3 weeks, fibers and fibrils within both groups became more homogenously organized and aligned, although UL tendons were not able to reach the same organizational structure as FL tendons. As opposed to FL tendons, stumps in UL tendons remained clearly distinguishable from the surrounding callus at the fibril level (Fig 1C). UL tendons also showed a delayed regeneration of fibril structure within the callus as d-spacing restoration was delayed (Fig 1D).

Discussion

Reduced i*n vivo* loading resulted in a delayed and more disorganized regeneration of the collagen structure within the callus of healing tendons. Additionally, unloading seems to delay remodeling of the stumps during early healing, postponing the callus tissue to merge with the stumps as well as the maturation of the callus tissue. These structural effects due to unloading would strongly affect mechanical properties of the tendon tissue and could be one reason behind the impaired mechanical competence following immobilization during tendon healing [1].

References

[1] Notermans et al., Eur Cell Mater 2021. [2] Hammerman et al., PLoS One, 2018. [3] Liebi et al., Nature, 2015. [4] Silva Barreto et al., Mat Biol, 2022. [5] Krause et al., Nat Med, 2010.

Acknowledgements

Funding from the Knut and Alice Wallenberg Foundation and European Research Council (No 101002516). Paul Scherrer Institut, Switzerland for beamtimes at cSAXS and TOMCAT.

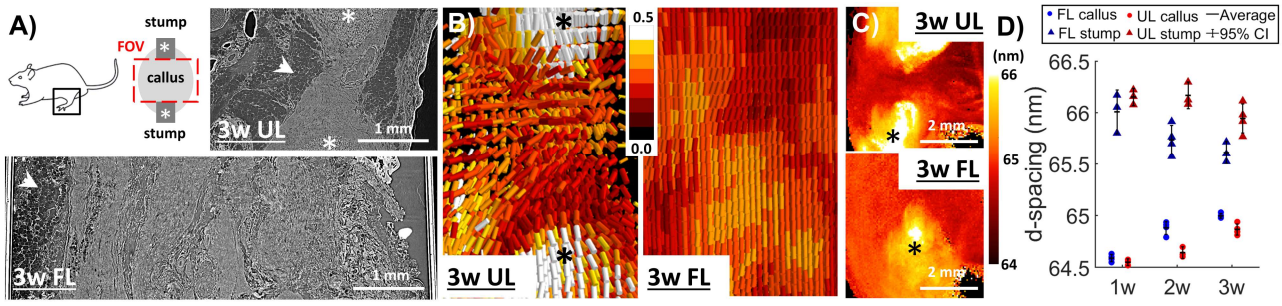

Fig 1. A) *Microscale organization obtained by PhC-µCT (* = stump, arrowhead = adipose tissue) and* ***B)*** *nanoscale organization obtained by SASTT (number of glyphs = fibril amount, glyph direction = fibril orientation, glyph colour = degree of fibril orientation) in the centre of a 3-weeks fully loaded (FL) and unloaded (UL) healing tendon.* ***C)*** *Representative distribution of fibril d-spacing obtained by 2D SAXS around the stumps in 3 weeks.* ***D)*** *Evolution of average fibril d-spacing obtained by 2D SAXS in the centre of the callus and stumps across healing time.*