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Multimodal characterization of heterotopic ossification during Achilles tendon healing in a rat animal model

Introduction: Clinical case studies of healing Achilles' tendons identified localized mineral depositions radiographically, described as 'heterotopic ossifications' (HO), or 'bone-like' tissue. The presence of HO has been associated with increased risk of re-ruptures and pain 1. The origin of HO in healing tendons is not yet fully understood. This study characterizes elementally and structurally the HO in healing tendons at the nanoscale.

Methods: Rat Achilles' tendons of Sprague-Dawley rats (10-14 weeks old) were transected and allowed to heal [2] for 3- and 12-weeks (N=3, N=2, respectively). The healing tendons were fixed, embedded in PMMA, and sectioned to 5m. Simultaneous micro-X-ray fluorescence (XRF) and diffraction (XRD) were conducted at I-18, Diamond Light Source (12.0keV, 2 x 2µm2 step size). Structural properties of HO were calculated by the Scherrer equation to yield the L- and W-parameters correlating to crystallite size along the c-axis, and the ab-plane respectively [3].

Results: Calcium (Ca) content is increasing between time points. Higher localized amounts of Zinc (Zn) and Iron (Fe) were present at the edges of HO preceding both higher Ca, and crystallite dimensions (L- and W-parameters). Zn was also observed around larger voids at 12-weeks, comparable visually to calcaneal bone, indicating ongoing remodelling.

Discussion: Spatial distribution of Zn is consistent with the mineralization process seen in embryonic mice [4]. Our results provide insight into key performers in the process of tendon tissue mineralization, and aid in the understanding of HO formation during the Achilles tendon healing process.

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