Title: Structural and immunological changes during spontaneous healing in Achilles tendons mice models.

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Introduction:

Recovery after tendon injuries remains a challenge for the orthopaedics to be solved due to the frequently poor clinical outcomes (1,2). To date, the cellular implications as well as the structural changes in tendinopathy are not fully known, especially concerning the link between inflammation and the early stages of tendinopathy (3). The aim of this research was to assess the structural changes and immune response that occur during spontaneous tendon healing in mice models.

Methodology:

Animal experimentation was authorised by the Italian Ministry of Health (183/2021). Under general anaesthesia, a bilateral incision of approximately 0.5 mm was induced on mice Achilles tendons. Healthy mice tendons were used as control. After 28 days postoperative, samples were used to perform Hematoxylin-Eosin, Alizarine Red staining, and immunohistochemistry (IHC) assays to assess the expression of collagen type 1 (COL1), collagen type 3 (COL3), von Willebrand Factor (FvW, an endothelial cell marker) and immune markers such as CD68 (pan-macrophage marker), CD86 (M1 macrophage), CD206 (M2 macrophage) markers. The samples were examined under light and fluorescence microscopes.

Results:

To understand the structural changes that occur during spontaneous tendon healing, the diseased mice tendon was compared to the healthy tendon tissue structure. Healthy tendons are characterized by hypocellularity, low vascularity and COL1 fibres parallelly organized along the longitudinal axis of the tissue. Furthermore, IHC demonstrated the absence of the immune cells markers within the healthy tendon. However, during spontaneous tendon healing, a disorganized hypercellularity was noted within the lesioned tendons accompanied by the presence of chondrocyte-like cells surrounded by a basophilic structure. The presence of chondrocytes was then confirmed by performing Alizarine Red staining from which chondrocyte-specific structures were identified, demonstrating the presence of calcium deposits

around the chondrocytes and throughout the lesioned areas. Additionally, there was a neo-formation of irregular collagen fibres, characterized by a predominant COL3 expression compared to COL1 during the early stage of spontaneous tendon healing, demonstrating an incomplete substitution of COL3 by COL1.Moreover, blood vessels within the healing tendon were assessed by analysing FvW. Compared to the healthy tendon, an increased number of blood vessels irregularly distributed was observed, especially within calcification areas. Finally, the analysis of immune markers, CD68, CD86, and CD206, revealed the presence macrophages within the lesioned tendon tissues.

Conclusions:

The preliminary results demonstrated structural differences between healthy and spontaneous tendon healing tissues. The majority of the spontaneous healing tendon samples presented chondrocyte-like cells and different stages of calcification processes, as well as major structural alteration, which might confirm that spontaneous tendon healing does not offer a full recovery and regeneration of tendon tissue. These results will be confirmed by analyzing the gene expression profiles of tendon and immunomodulatory genes and will be further compared with tendon healing after stem cell transplantation.

Acknowledgement: This research is part of the P4 FIT project ESR 13, funded under the H2020-ITN-EJD MSCA grant agreement No.955685.

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