Higher Efficiency Microdamage Clearance in MRL/MpJ Tendons Following Laser Ablation

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INTRODUCTION: Tendinopathy is a common pathology that affects 10% of adults under the age of 45 and results in pain, degradation of tendon structure, and diminished tissue function [1]. Despite well-established treatments, repaired tendons are characterized by the formation of scar tissue and diminished mechanical properties [1]. While most mammalian tissue regeneration is limited, the Murphy Roths Large (MRL/MpJ) mouse can regenerate multiple tissues, including tendon [2]. In tendon, improved regeneration is marked by superior matrix synthesis, matrix organization and cell proliferation, as well as the return of mechanical function similar to uninjured tendon [3]. Previously, we showed that this regenerative capacity of MRL/MpJ tendons may stem from the MRL/MpJ tenocyte's increased capacity for matrix remodeling, defined by greater matrix turnover in response to stress deprivation [4]. However, it remains unclear if the MRL/MpJ tenocytes heightened remodeling response extends beyond mechanical injury to physical injuries such as matrix damage. Therefore, the purpose of this study was to use our established novel laser ablation model to induce localized microdamage to live tendon explants and characterize the remodeling response of MRL/MpJ tenocytes. We hypothesized that resident MRL/MpJ tenocytes would have the ability to initiate remodeling more rapidly compared to C57BL/6J (B6) tenocytes, resulting in faster clearance of matrix microdamage generated by laser ablation.

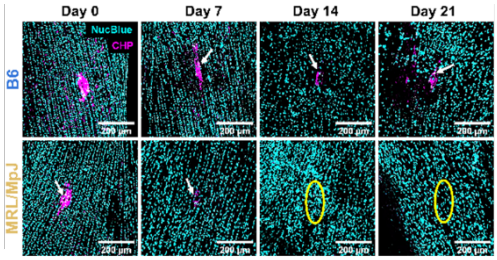

Figure 1: Representative max intensity projection images of collagen damage (magenta) and cell nuclei (cyan). Damage in B6 explants (top row) and MRL/MpJ explants (bottom row) at days 0, 7, 14, and 21. White arrow highlights area CHP stain around cells. Yellow ellipse highlights area of ablation damage.

METHODS: Flexor digitorum longus (FDL) tendon explants were harvested from mature C57BL/6J (B6) and MRL/MpJ mice at 8 weeks of age (BU IACUC Approved), as described previously [5]. FDL explants were then loaded into custom grips with a gauge length of 10 mm. Samples were transferred to culture wells in a custom-built imaging bioreactor and standard culture media was added to each well [6]. Tendons were hand tensioned using a custom tensioning bar until taut. The bioreactor was then transported to the stage of a Zeiss Axiovert S100 microscope equipped with a 10x objective and a 1,064-nm pulsed Nd:YAG nanosecond laser (Minilite, Continuum). The midportion of the tendon was ablated using a single laser pulse of 2 mJ. Explants were then cultured in place for up to 21 days. Collagen damage and cell location were determined by Cy3 Collagen Hybridizing Peptide (CHP) and NucBlue nuclear fluorescent stains, respectively, at day 0, 7, 14, and 21. Confocal image z-stacks of the ablation damage and hole were then captured on an Olympus FV3000 with a 20x objective. The depth of the damage was assessed by identifying the number of z-stacks containing CHP stain and multiplying by the distance between z-stacks. The volume of damage was determined using an ROI that traced the area of CHP stain and the voxel counter plugin in ImageJ. Cells in the damaged area were determined by counting the number of cells in the NucBlue channel that fell within the traced ROI. Average collagen damage intensity was determined from a max intensity projection of the CHP channel using the same traced ROI. Tendons were also categorized based on their appearance at the end of culture. If the tendon still had CHP stain it was categorized as 'damaged', if it had no CHP stain it was categorized as 'cleared', and if it had widespread disrupted matrix it was categorized as 'degenerated'. Statistical evaluation was performed using main effects two-way ANOVAs with post-hoc Bonferroni corrected t-tests. Significance was noted at $p<0.05$ and is denoted with a bar (-).

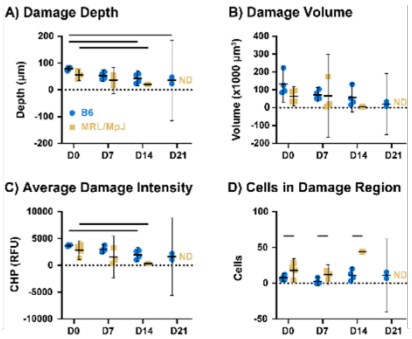

Figure 2: (A) Average intensity, (B) peak intensity, (C) volume, and (D) cell count of damaged area for B6 (blue) and MRL/MpJ (yellow). Data is mean ± 95% confidence interval. 'ND' indicates no CHP stain was found at the ablation site and therefore no data is available.

RESULTS: Representative images show a progressive reduction in CHP stain around the ablated hole during the culture period for both B6 and MRL/MpJ explants, but only the MRL/MpJ explants are able to fully clear the denatured collagen marked by CHP (Fig. 1). For both groups we found a significant reduction in depth of CHP stain into the tendon by day 14 and a continued reduction in signal depth to day 21 in the B6 group (Fig. 2A). This was accompanied by a reduction in the damage volume throughout culture in both B6 and MRL/MpJ explants (Fig. 2B). We also found a significant reduction in the average intensity of collagen damage from days 0 to 14 (Fig. 2C). There were no differences in cell number within the damage area until over time in either group, but the number of cells in the damaged region were significantly greater at days 0,7, and 14 in the MRL/MpJ explants (Fig. 3D). Finally, we observed that as early as day 7 there is no visible CHP staining around the hole in some of the MRL/MpJ explants (Fig. 3). In both groups, we also observed that some explants degenerated by the end of culture with widespread disruption to the collagen fibers.

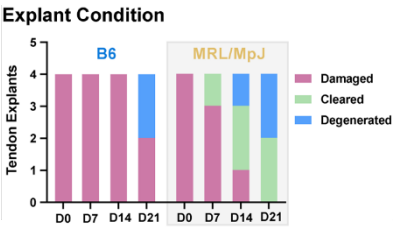

Figure 3: Condition of the B6 (left) and MRL/MpJ (right) explants at each day of culture. Explants categorized as damaged, cleared, or degenerated.

DISCUSSION: Our data demonstrate that the heightened remodeling capacity of the MRL/MpJ tenocytes extends to physical injuries such as matrix microdamage. Specifically, MRL/MpJ tenocytes can initiate clearance of localized matrix microdamage more rapidly, leading to full clearance of denatured collagen as early as 7 days after damage. The reduction in damage depth and volume demonstrates that this is not a superficial process occurring at the surface of the tendon. The removal of stain is likely to be an active process by resident cell types, such as fibroblast-like tenocytes or a recently identified macrophage-like cell population, known as tenophages [7]. This suggests that the greater number of cells localized to the microdamage in the MRL/MpJ tendons throughout culture may be vital in their ability to clear denatured collagen more rapidly. Both tenocytes and tenophages can produce the collagen type I degrading matrix metalloproteinase MMP-9 [8], which has been shown to selectively remove denatured collagen in tendons with microdamage [9]. We also suspect that the ability of resident MRL/MpJ tendon cells to more rapidly clear matrix microdamage stems from their capacity to produce collagen degrading MMPs more efficiently than B6 tenocytes [2,4,10]. While this greater clearance of collagen may have been beneficial in removing denatured collagen at the damage site, it may have also led to the eventual degeneration of some of the explants later in culture. Future studies will explore the local expression of matrix degrading enzymes and their inhibitors around the injury site to identify mechanisms and cellular drivers of enhanced denatured collagen removal in MRL/MpJ explants and degeneration seen in both B6 and MRL/MpJ explants. Regardless, this study demonstrates a more rapid response to localized microdamage injury in MRL/MpJ tendons, suggesting mechanisms of MRL/MpJ matrix remodeling in response to matrix damage may also be innately different.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates the utility of the MRL/MpJ model in elucidating mechanisms of enhanced matrix remodeling and sheds light on its potential to help identify local tendon-specific factors that can be leveraged for therapeutics.

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